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PROCEEDINGS

OF THE

NEW YORK PATHOLOGICAL SOCIETY

NEW SERIES, VOLUME XV

1915

ORGANIZED IN 1844

INCORPORATED IN 1886

NEW YORK
1915

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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LIST OF OFFICERS AND COMMITTEES FOR THE YEAR 1915

President:
DR. HANS ZINSSER

Vice-President:
DR. ROBERT A. LAMBERT

Secretary and Editor:
DR. L. W. FAMULENER

Treasurer:
DR. F. C. WOOD

Committee on Admission and Ethics:
DR. HARLOW BROOKS
DR. CYRUS W. FIELD
DR. CHARLES NORRIS
DR. AUGUSTUS B. WADSWORTH

Committee on Publication:
DR. W. G. MACCALLUM
DR. ALWIN PAPPENHEIMER
DR. F. C. WOOD, *Treasurer*
DR. L. W. FAMULENER, *Secretary and Editor*

Committee on Microscopy:
DR. JAMES EWING
DR. CHARLES NORRIS
DR. F. C. WOOD

PRESIDENTS OF THE SOCIETY.

*Dr. John A. Sweet	1844
*Dr. Willard Parker	1845, 1846, 1847
*Dr. James R. Wood	1848, 1857
*Dr. T. M. Markoe	1849, 1879
*Dr. W. H. Van Buren	1850
*Dr. Charles E. Isaacs	1851
*Dr. John T. Metcalfe	1852
*Dr. Henry Van Arsdale	1853
*Dr. James Bolton	1854, 1855
*Dr. Robert Watts	1856
*Dr. Edmund R. Peaslee	1858
*Dr. John C. Dalton	1859
*Dr. Alfred C. Post	1861
*Dr. Thomas C. Finnell	1862
*Dr. David S. Conant	1863
Dr. Abraham Jacobi	1864
*Dr. Gurdon Buck	1865
*Dr. Henry B. Sands	1866
*Dr. William B. Bibbins	1867
*Dr. Ernest K. Krackowitzer	1868
*Dr. L. A. Sayre	1869
*Dr. Joseph C. Hutchinson	1870
*Dr. A. L. Loomis	1871, 1872
*Dr. Erskine Mason	1873
*Dr. Herman Knapp	1874
*Dr. Francis Delafield	1875
Dr. Charles K. Briddon	1876
*Dr. Edward G. Janeway	1877
*Dr. John C. Peters	1878
Dr. Edward L. Keyes	1879
Dr. T. E. Satterthwaite	1880, 1881
*Dr. E. C. Seguin	1882
*Dr. George F. Shrady	1883, 1884
Dr. John A. Wyeth	1885, 1886
Dr. T. M. Prudden	1887, 1898, 1899

* Deceased.

Dr. W. P. Northrup	1888, 1889
*Dr. J. West Roosevelt	1890
Dr. Hermann M. Biggs	1891
*Dr. H. P. Loomis	1892, 1893
*Dr. G. C. Freeborn	1894
Dr. George P. Biggs	1895
*Dr. John Slade Ely	1896, 1897
*Dr. Eugene Hodenpyl	1900
Dr. Edward K. Dunham	1901
Dr. James Ewing	1902
Dr. William H. Park	1903
Dr. O. H. Schultze	1904
Dr. Harlow Brooks	1905
Dr. F. S. Mandlebaum	1906
Dr. Charles Norris	1907
Dr. E. Libman	1908
Dr. Horst Oertel	1909
Dr. Richard M. Pearce	1910
Dr. W. G. MacCallum	1911, 1912
Dr. A. B. Wadsworth	1913
Dr. Alwin M. Pappenheimer	1914
Dr. Hans Zinsser	1915

SECRETARIES.

*Dr. W. C. Roberts	1844 to 1849
*Dr. H. D. Buckley	——
*Dr. Henry G. Cox	1850 to 1852
*Dr. William H. Church	1852
*Dr. Chas. M. Allen	1852 to 1853
*Dr. Geo. T. Elliot	1853 to 1854
*Dr. J. F. Jenkins	1854 to 1855
*Dr. E. Lee Jones	1855 to 1861
*Dr. T. G. Thomas, *pro tem*	1855
*Dr. H. D. Noyes, *pro tem*	1858
*Dr. Geo. F. Shrady	1861 to 1879
*Dr. W. M. Carpenter	1880 to 1888
Dr. Walter Mendelson	1888

* Deceased.

Dr. T. L. Stedman	1889 to 1891
Dr. Ogden C. Ludlow	1891 to 1902
Dr. F. C. Wood	1902 to 1907
Dr. Karl M. Vogel	1907 to 1910
Dr. Alwin M. Pappenheimer	1911 to 1914
Dr. Robert A. Lambert	1914
Dr. L. W. Famulener	1915

LIFE MEMBERS OF THE SOCIETY

Elected		
1877	Abbe, Robert	13 West 50th Street
1889	Carter, De Lancy	118 East 85th Street
1887	Cheesman, Timothy M.	Garrison-on-Hudson, New York
1884	Dixon, George A.	15 West 49th Street
1889	Dunham, Edward K.	35 East 68th Street
1855	Eliot, Ellsworth	34 East 67th Street
1853	Emmet, Thomas Addis	89 Madison Avenue
1888	Freeman, Rowland G.	211 West 57th Street
1874	Gibney, Virgil P.	16 Park Avenue
1889	Grauer, Frank	226 West 71st Street
1887	Hamilton, Charles S.	150 East Broad St., Columbus, Ohio
1882	Holt, L. Emmett	14 West 55th Street
1880	Jackson, Frank W.	555 Madison Avenue
1861	Jacobi, Abraham	19 East 47th Street
1887	James, Walter B.	7 East 70th Street
1879	Judson, A. B.	53 Washington Square
1871	Leale, Charles A.	604 Madison Avenue
1883	LeBoutillier, William G.	Long Lake, Hamilton Co., N. Y.
1880	Lewis, Daniel	616 Madison Avenue
1885	Lockwood, George Roe	18 East 52d Street
1889	MacHale, Ferdinand	317 East 87th Street
1878	McNutt, Sarah J.	265 Lexington Avenue
1878	Mayer, Abraham	40 East 60th Street
1886	Meyer, Willy	700 Madison Avenue
1874	Moeller, Henry	140 West 88th Street
1883	Northrup, William P.	57 East 79th Street
1858	Packard, Charles W.	455 Park Avenue
1886	Peterson, Frederick	20 West 50th Street
1874	Polk, William M.	310 Fifth Avenue

Elected		
1878	Porter, William H.	46 West 83d Street
1883	Prudden, T. Mitchell	160 West 59th Street
1884	Rice, Clarence C.	123 East 19th Street
1881	Robinson, A. R.	159 West 49th Street
1874	Satterlee, F. LeRoy	6 West 56th Street
1872	Satterthwaite, Thomas E.	7 East 80th Street
1887	Sayre, R. H.	14 West 48th Street
1890	Seaman, Louis L.	247 Fifth Avenue
1872	Stimson, Daniel M.	28 West 37th Street
1886	Thacher, John S.	20 West 50th Street
1888	Van Cott, J. M.	188 Henry Street, Brooklyn
1878	Walker, Henry F.	18 West 55th Street
1882	Wiener, R. G.	48 East 65th Street
1885	Wright, Jonathan,	Windy Rock, Westchester Co., Pleasantville, N. Y.
1881	Wylie, W. Gill	28 West 40th Street

MEMBERS OF THE SOCIETY

Elected		
1898	Adler, Isaac	401 West End Avenue
1914	Baehr, George	51 East 96th Street
1898	Bailey, Frederick R.	1465 N. J. Avenue, Elizabeth, N. J.
1907	Bang, Richard T.	139 West 11th Street
1903	Bartlett, C. J.	183 Bishop Street, New Haven, Conn.
1902	Bastedo, Walter A.	57 West 58th Street
1892	Beach, Bennett S.	144 West 85th Street
1905	Beer, Edwin	48 West 74th Street
1900	Berkeley, W. N.	133 East 61st Street
1913	Bernstein, Harry S.	Health Department, Providence, R. I.
1891	Biggs, George P.	133 West 71st Street
1901	Bishop, Louis F.	54 West 55th Street
1911	Boehm, Emil	3806 Delmar Boulevard, St. Louis, Mo.
1905	Bolduan, Charles F.	139 Centre Street
1896	Bovaird, David	137 East 60th Street
1901	Brill, N. E.	48 West 76th Street
1896	Brooks, Harlow	44 West 9th Street
1908	Buerger, Leo	40 East 41st Street
1913	Burrows, M. T.,	Johns Hopkins Medical School, Baltimore, Md.

Elected		
1911	Butterfield, E. E.	135 East 34th Street
1915	Cary, Edward G.	1145 Amsterdam Avenue
1910	Cecil, Russell L.	123 East 62d Street
1908	Celler, Herbert L.	61 West 94th Street
1913	Coca, A. F.	342 State Street, Flushing, N. Y.
1909	Cohn, A. E.	316 Central Park West
1910	Cole, Rufus	960 Park Avenue
1891	Coleman, Warren	58 West 55th Street
1893	Coley, William B.	521 Park Avenue
1903	Collins, Katherine R.	State Board of Health, Atlanta, Ga.
1914	Cornwall, Leon H.	375 Park Avenue
1896	Denton, Myron B.	129 East 30th Street
1914	Diner, Jacob	316 West 84th Street
1913	Eisenbrey, A. B.	St. Luke's Hospital
1910	Elsberg, C. A.	Madison Avenue and 63d Street
1901	Elser, William J.	Cornell Med. Col., 1st Ave. and 28th St.
1915	Evans, Frank A.	Presbyterian Hospital
1908	Ewald, Louis A.	48 East 87th Street
1893	Ewing, James	264 West 57th Street
1909	Famulener, L. W.	St. Luke's Hospital
1913	Felberbaum, David	561 West 163d Street
1901	Field, Cyrus W.	24 East 48th Street
1905	Flexner, Simon	Rockefeller Inst., Avenue A and 66th St.
1892	Fordyce, John A.	8 West 77th Street
1912	Fox, Fred J.	124 East 19th Street
1905	Frank, Robert T.	983 Park Avenue
1915	Fraser, Alexander	601 West 148th Street
1914	Fried, G. A.	315 Central Park West
1913	Garside, C. Z.	Hotel Bretton Hall, Bway. and 86th Street
1903	Goodman, A. L.	136 West 87th Street
1914	Gutherson, W. F.	37 Hamilton Street, Paterson, N. J.
1908	Haas, Sidney V.	666 West End Avenue
1911	Hall, Morton E.	11125 89th Ave., Edmonton, Alberta, Can.
1912	Harvey, S. C.	Peter Bent Brigham Hospital, Boston, Mass.
1905	Hastings, Thomas	22 East 68th Street
1907	Hess, Alfred F.	16 West 86th Street
1912	Hillman, Oliver	214 West 110th Street
1915	Holt-Harris, J. E.	Quarantine Station, Rosebank, N. Y.

Elected		
1911	Hopkins, J. G.	350 Washington Ave., Brooklyn
1893	Huddleston, John H.	145 West 78th Street
1911	Huffman, Otto V.	89 Joralemon Street, Brooklyn
1912	Humphreys, Frederick B.	630 West 141st Street
1911	Huntoon, F. M.	Cornell Med. Col., First Ave. and 28th St.
1894	Jeffries, Ferdinand	341 West 50th Street
1900	Jessup, D. S. D.	601 West 113th Street
1913	Jobling, J. W.	Vanderbilt University, Nashville, Tenn.
1903	Jones, S. Fosdick	250 Metropolitan Building, Denver, Colo.
1909	Kast, Ludwig	771 Madison Avenue
1903	Keller, F. C.	77 West 50th Street
1910	Kessel, Leo	88 Central Park West
1915	Kline, Benjamin S.	Montefiore Hospital, N. Y.
1891	Kneer, Ferdinand G.	236 West 51st Street
1908	Kohn, Albert	122 East 58th Street
1910	Krumwiede, C.	15 Hobart Street, Bronxville, N. Y.
1912	Lamb, Albert	171 East 71st Street
1893	Lambert, Alexander	43 East 72d Street
1912	Lambert, R. A.	735 Park Avenue
1899	Langmann, Gustav	121 West 57th Street
1907	Lapowski, Boleslaw	180 West 58th Street
1896	Larkin, John H.	City Hospital
1904	Leale, Medwin	852 Lexington Avenue
1912	L'Esperance, Elise	Cornell Med. Col., 1st Ave. and 28th St.
1898	Levin, Isaac	119 West 71st Street
1915	Levy, I. J.	76 West 86th Street
1908	Lewisohn, Richard	565 Park Avenue
1899	Libman, Emanuel	180 East 64th Street
1908	Lindsay, Gordon	1337 71st Street, Brooklyn
1911	Longcope, Warfield T.	116 East 58th Street
1915	Long, W. Bayard	1257 Washington Avenue
1891	McAlpin, D. Hunter	68 William Street
1910	McDonald, Ellice	174 West 58th Street
1900	McLaughlin, George E.	41 Crescent Avenue, Jersey City
1909	MacCallum, W. G.	437 West 59th Street
1912	MacNeal, Ward J.	303 East 20th Street
1905	Mandel, Arthur R.	150 East 40th Street
1895	Mandlebaum, F. S.	1300 Madison Avenue

Elected
1901 Manges, M..........................72 East 79th Street
1900 Mannheimer, George.................41 West 51st Street
1899 Markoe, James W...................20 West 50th Street
1909 Martland, H. S..........1138 Broad Street, Newark, N. J.
1901 Meltzer, S. J...Rockefeller Institute, Avenue A and 66th St.
1898 Moschcowitz, Alexis V..............925 Madison Avenue
1904 Moschcowitz, Eli......................41 West 83d Street
1903 Murray, Archibald...................36 Gramercy Park
1892 Myles, Robert C.......................46 West 38th Street
1894 Norrie, Van Horne...................21 West 37th Street
1896 Norris, Charles.........................Bellevue Hospital
1895 Noyes, William B....................221 West 57th Street
1899 Oertel, Horst...Royal Victoria Hospital, Montreal, Canada
1914 Olcott, G. P., Jr.........................772 Park Avenue
1906 Oppenheimer, B. S..................125 West 86th Street
1915 Ottenberg, Reuben..................15 West 89th Street
1905 Pappenheimer, A. M.................437 West 59th Street
1893 Park, William H...................315 West 76th Street
1903 Patterson, Henry S.................130 East 62d Street
1909 Pease, Herbert D....................39 West 38th Street
1913 Petersen, William F..Vanderbilt University, Nashville, Tenn.
1898 Phillips, Carlin......................166 West 72d Street
1900 Potter, N. B...............................591 Park Avenue
1912 Ray, Edgar T.................824 St. Nicholas Avenue
1914 Riley, E. J..........................605 West 181st Street
1915 Riley, Henry A......................437 West 59th Street
1914 Rohdenberg, G. L................222 West 136th Street
1891 Sachs, B...........................116 West 59th Street
1902 Satterlee, G. R.....................125 West 58th Street
1900 Schlapp, Max.........................40 East 41st Street
1897 Schley, W. S..........................24 West 45th Street
1915 Schöhl, Otto.........Quarantine Station, Rosebank, N. Y.
1897 Schultze, Otto H.....................47 East 58th Street
1910 Schwartz, Benjamin.......................261 7th Street
1903 Schwarz, Herman.....................50 East 91st Street
1895 Shelby, Edmund P., Jr...............116 West 74th Street
1913 Sittenfield, M. J....................437 West 59th Street
1899 Smith, Ernest E..........................50 E. 41st Street
1894 Sondern, Frederic E................200 West 56th Street

Elected		
1908	Stein, Arthur P.	11 East 68th Street
1898	Stein, Richard	79 East 79th Street
1901	Steinhardt, E. R.	Research Laboratory, East 16th St.
1911	Stillman, Charles K.	Mystic, Conn.
1913	Stillman, E. G.	17 East 72d Street
1911	Strong, L. W.	Woman's Hospital
1912	Symmers, Douglas	Bellevue Medical School
1912	Taylor, Richard	303 East 20th Street
1915	Teague, Oscar	Quarantine Station, Rosebank, N. Y.
1913	Terry, B. T.	King's County Hospital, Brooklyn
1913	Thalhimer, William	86 West 83d Street
1913	Tytler, W. H.	66 Paisley Street, Guelph, Ont.
1905	Van Beuren, Frederick T., Jr.	812 Park Avenue
1912	Vance, Morgan	Bellevue Hospital
1905	Vogel, Karl M.	680 Madison Avenue
1897	Wadsworth, A. B.	State Dept. of Health, Albany, N. Y.
1911	Wallace, Guy H.	Bellevue Hospital
1907	Ward, Wilbur	24 West 50th Street
1905	Warren, Mortimer	24 Gramercy Park
1904	Weil, Richard	970 Park Avenue
1903	Welch, J. E.	138 West 70th Street
1903	Welt-Kakels, Sara	35 East 61st Street
1908	Wiener, Solomon	67 West 89th Street
1907	Wilcox, Herbert B.	159 East 70th Street
1894	Williams, Anna W.	549 Riverside Drive
1901	Wilson, R. J.	549 Riverside Drive
1913	Woglom, William H.	110 Morningside Drive
1891	Wollstein, Martha	1 West 81st Street
1898	Wood, Francis Carter	1145 Amsterdam Avenue
1912	Youland, W. E.	123 State Street, Albany, N. Y.
1905	Zinsser, Hans	437 West 59th Street

CERTIFICATE OF INCORPORATION.

State of New York,
City and County of New York, } *ss.:*

We, the subscribers, who are severally members of the unincorporated society known as the New York Pathological Society, founded in the City of New York in the year one thousand eight hundred and forty-four, namely:—

Alonzo Clark,
Middleton Goldsmith,
John C. Peters,
Lewis A. Sayre,
Thomas M. Markoe,
Gustavus A. Sabine,
John C. Dalton,
Abraham Jacobi,
Alfred L. Loomis,
Francis Delafield,
Edward G. Janeway,
George F. Shrady,
Wesley M. Carpenter,
John A. Wyeth,
Willard Parker,
Thomas Addis Emmet,
T. Mitchell Prudden,
William P. Northrup,
Louis Waldstein,
L. Emmett Holt,
John H. Hinton,
George L. Peabody,
Thos. E. Satterthwaite,

who are severally Doctors of Medicine, and who severally reside in the City, County, and State of New York, and are citizens thereof, except the above-named Middleton Goldsmith, who resides in Rutland, in the State of Vermont, and is a citizen thereof,

Do hereby certify that we desire to form a Corporation under and in pursuance of Chapter 319 of the Laws of 1848, of the State of New York, entitled "An Act for the incorporation of benevolent, charitable, scientific, and missionary societies," and the several acts amending and extending the same; particularly Chapter 526 of the Laws of 1881, passed June 15, 1881; and we therefore hereby state:—

1. The name of the said Corporation by which it shall be known in law shall be the "New York Pathological Society."

2. The particular business and objects of such society are the improvement of its members in Pathology and in Diagnosis and Treatment of Diseases as founded upon Pathology.

3. The number of Trustees, Directors, or Managers to manage

the same shall be six; and their names for the first year of its existence are:—

John C. Peters, M.D.,
T. Mitchell Prudden, M.D.,
William P. Northrup, M.D.,
Louis Waldstein, M.D.,
L. Emmett Holt, M.D., and
John H. Hinton, M.D.

4. The office and principal place of business of said Corporation shall be in the City, County, and State of New York.

Witness our hands and seals, this first day of April, one thousand eight hundred and eighty-six.

A. Clark [L.S.]
Gustv. A. Sabine [L.S.]
John C. Peters [L.S.]
Willard Parker [L.S.]
Wesley M. Carpenter [L.S.]
J. A. Wyeth [L.S.]
T. E. Satterthwaite [L.S.]
J. C. Dalton [L.S.]
L. Emmett Holt [L.S.]
Francis Delafield [L.S.]
George F. Shrady [L.S.]
L. Waldstein [L.S.]
Lewis A. Sayre [L.S.]
Wm. P. Northrup [L.S.]
Alfred L. Loomis [L.S.]
T. Mitchell Prudden [L.S.]
George L. Peabody [L.S.]
A. Jacobi [L.S.]
Thos. Addis Emmet [L.S.]
T. M. Markoe [L.S.]
John H. Hinton [L.S.]
Edward G. Janeway [L.S.]

City and County of New York, ss.:

I hereby certify that, on the respective days hereinafter specified, before me personally came the following-named individuals, respectively; that is to say, on the first day of April, A.D. 1886:

Alonzo Clark,
Gustavus A. Sabine,
John C. Peters,
Willard Parker,
Wesley M. Carpenter,
John A. Wyeth,
Thos. E. Satterthwaite,
John C. Dalton,
L. Emmett Holt,
Thomas Addis Emmet,
Thomas M. Markoe,
Francis Delafield,
George F. Shrady,
Louis Waldstein,
Lewis A. Sayre,
William P. Northrup,
Alfred L. Loomis,
T. Mitchell Prudden,
George L. Peabody,
Abraham Jacobi;

and on the second day of April, A.D. 1886:

John H. Hinton and Edward G. Janeway.

who are severally personally known to me, and to me severally known to be the individuals described in and who executed the foregoing Certificate and they severally acknowledged to me that they executed the same for the purpose therein mentioned.

Witness my hand and seal, this second day of April, 1886.

S. B. GOODALE,
[L.S.] (99) *Notary Public,*
N. Y. County.

I approve of the within Certificate, and consent to the filing of the same.

New York, April 5th, 1886. C. DONOHUE,
Justice of the Supreme Court.

State of New York,
City and County of New York, } *ss.:*

I, JAMES A. FLACK, Clerk of the said City and County, and Clerk of the Supreme Court of said State for said County, do certify, that I have compared the preceding with the original Certificate of Incorporation of

The New York Pathological Society

on file and recorded in my office, and that the same is a correct transcript therefrom and of the whole of such original.

Indorsed, filed, and recorded April 5th, 1886.

In witness whereof I have hereunto subscribed my name and affixed my seal, this 5th day of April, 1886.

[L.S.] JAMES A. FLACK.

State of New York,
Office of the Secretary of State, } *ss.:*

I have compared the preceding with the original Certificate of Incorporation of the "New York Pathological Society," with acknowledgment thereunto annexed, filed, and recorded in this office on the sixth day of April, 1886, and do hereby certify the same to be a correct transcript therefrom and of the whole of the said original.

[L.S.] Witness my hand and the seal of the office of the Secretary of State, at the City of Albany, this sixth day of April, one thousand eight hundred and eighty-six.

FREDERICK COOK,
Secretary of State.

State of New York,
Office of Secretary of State, } *ss.:*
Filed and recorded April 6th, 1886.

DIEDRICH WILLERS,
Deputy Secretary of State.

CONSTITUTION.

ARTICLE I.

NAME, MEMBERS.

This Society, now duly incorporated under the laws of the State of New York, shall be known as the NEW YORK PATHOLOGICAL SOCIETY, and shall consist of active and honorary members.

ARTICLE II.

OBJECTS.

Its objects shall be the advancement of the knowledge of Pathological Anatomy, Histology, and General Pathology, and the improvement of its members in these departments, and in the diagnosis and treatment of disease as founded upon them.

ARTICLE III.

OFFICERS.

Its officers shall be a President, a Vice-President, a Secretary, a Treasurer, and an Editor of the Transactions, whose terms of office shall be one year; and six Trustees, two of whom shall be elected each year to serve for three years.

ARTICLE IV.

THE PRESIDENT.

The President, or, in his absence, the Vice-President, or, in his absence, a Chairman *pro tempore,* shall preside at all meetings of the Society, and shall have a casting vote. He shall preserve order, and shall decide all questions of order, subject to an appeal to the Society. He shall also appoint all Committees authorized by the Society, unless otherwise specially ordered.

ARTICLE V.

THE SECRETARY.

The Secretary shall preserve minutes of the proceedings of the Society, and shall receive all histories, reports of Committees, and illustrations of specimens presented, and shall deliver the same into the hands of the Editor of the Transactions within six weeks after their approval. He shall issue notices of the meetings of the Society, and notify, in writing, members-elect of their election, and of the requirements of the By-Laws.

ARTICLE VI.

THE EDITOR.

The Editor of the Transactions shall collate from the material specified in the preceding Article such portions as may appear to the Committee on Publication of sufficient value to merit publication, and shall arrange them for the press, but the Society shall decide upon their publication after the Editor shall have submitted the estimated cost to the Trustees.

ARTICLE VII.

THE TRUSTEES.

The Trustees shall have the control and management of the financial affairs and funds of the Society, and no measure involving the expenditure of money, other than for the current expenses, shall be valid without the approval of the majority of the Board.

The Trustees shall render the Society, at the anniversary meeting in each year, a detailed report of their proceedings, and a particular statement of all moneys due, received, and paid out during the immediately preceding year, and of all outstanding trusts and obligations of the Society.

The Trustees, by a majority vote, shall fill any vacancy that may arise in their number, for the unexpired portion of the term of office.

ARTICLE VIII.

THE TREASURER.

The Treasurer shall take in charge all funds and trusts; shall collect all dues and obligations, and may employ an agent for that purpose at the expense of the Society; shall keep an account of all receipts and disbursements, and shall pay all just demands; but in the performance of these and all other duties of his office he shall be under the direction of the Trustees, and shall make no permanent investments without the approval of the majority of the Board.

He shall report to the Society at the anniversary meeting in January, and also to the Trustees whenever requested by them. He shall also report, at said meeting, the names of all members in arrears of dues, with the respective amount of their dues.

He shall be a Trustee and also a member of the Committee on Publication.

ARTICLE IX.

COMMITTEES.

1. *Committee on Admission and Ethics.*—The Society shall annually elect, by ballot, five of its members as a Committee on Admission and Ethics, to which all nominations for membership and questions relating to ethics shall be referred.

2. *Committee on Publication.*—This Committee shall consist of the Editor, the Treasurer, the Secretary, and two other members of the Society elected annually by ballot. The above Committees shall report to the Society annually at the anniversary meeting.

3. *Committee on Microscopy.*—The President shall annually appoint a Committee on Microscopy, which shall consist of three members of the Society.

This Committee shall have the care of the microscope, specimens, and instruments belonging to the Society, and shall examine all specimens referred to it, and report thereon in writing to the Society within one month.

ARTICLE X.

ANNUAL ASSESSMENT.

Any money that may be necessary to defray the current annual expenses of the Society shall be raised by a tax upon the members, the amount upon each being recommended by the Treasurer and the Trustees, and sanctioned by a vote of the Society at the anniversary meeting in January.

ARTICLE XI.

AMENDMENTS.

This Constitution shall take effect immediately, and it shall not be altered except by a three-fourths vote of the active members present and voting at a stated meeting subsequent to one at which a proposition to that effect shall have been made in writing, and then only after the proposed alteration shall have been printed and sent to every active member of the Society.

BY-LAWS.

ARTICLE I.

MEETINGS.

The stated meetings of the Society shall be held at 8:30 o'clock on the evening of the second Wednesday of each month, except during the months of June, July, August, and September, when no meetings shall be held. Special meetings may be called by the Secretary, at the request of the President, or on a requisition signed by ten members.

ARTICLE II.

QUORUM.

Five members shall constitute a quorum for the ordinary business, and ten for the executive business of the Society.

ARTICLE III.

ORDER OF BUSINESS, STATED MEETING.

The order of business at the stated meetings shall be:—

1. Reading the minutes of the preceding meeting.
2. Report of the Committee on Microscopy.
3. Presentation of specimens with written or oral histories, with remarks thereon.

4. Executive Session at which shall be presented:
   a. The minutes of the last executive session.
   b. The Report of the Committee on Admission and Ethics.
   c. Any other executive business.
5. Adjournment.

ARTICLE IV.

ORDER OF BUSINESS, ANNIVERSARY MEETING.

The anniversary meeting shall be held on the second WEDNESDAY in January. The order of business at the executive session of this meeting shall be:—

1. Reading of the minutes of the last executive session.
2. Report of the Treasurer.
3. Report of the Editor and the Committee on Publication.
4. Report of the Committee on Admission and Ethics.
5. Election of the Officers, the Trustees, the Committee on Admission and Ethics, and the Committee on Publication.

ARTICLE V.

ELECTIONS.

1. All the Officers, the Trustees, the Committee on Admission and Ethics, and the Committee on Publication shall be elected by ballot and by a majority of the active members present and voting.

2. Two Trustees shall be elected annually for a period of three years at the anniversary meeting, according to the requirements of Article III of the Constitution, notice of which shall be given with the notice of the meeting.

ARTICLE VI.

REQUIREMENTS FOR ADMISSION.

The requirements for admission to membership in this Society shall be:—

1. That the applicant be a licensed physician in good standing or one who is engaged in fields of scientific work other than medicine, that is, those engaged in work in bacteriology, physiology, physiological chemistry, and other medical sciences.

2. That he present through a member, on printed forms prescribed by the Society, both an application for membership, and the

recommendation of three members of the Society, together with his diploma if he is not a member of either the Medical Society of the County of New York, or of the New York Academy of Medicine.

3. That he receive the assenting ballots of four-fifths of the members present.

ARTICLE VII.

MEMBERS ELECT.

A member elect shall not be entitled to the privileges of membership until he has paid the initiation fee; nor shall an election be valid longer than three months, unless these conditions are fulfilled. This shall not apply to honorary members.

ARTICLE VIII.

INITIATION FEE.

The initiation fee shall be five dollars. When a candidate shall be elected to membership of the Society at a meeting, within the calendar year, subsequent to the first meeting in October, the annual tax first required of such member shall be that of the year succeeding the year of such election.

ARTICLE IX.

RESIGNATIONS.

A resignation of membership shall not be accepted unless presented in writing, and after payment of all dues.

ARTICLE X.

REVISION OF CONTRIBUTIONS.

A member desiring to revise the history of his specimen, after it has been placed in the hands of the Editor, may receive it for that purpose, but must return the same to that officer within two weeks.

ARTICLE XI.

DUES.

The annual dues of active members shall be five dollars. The payment of one hundred dollars at one time shall exempt a member from the payment of further dues. Those who have been members for twenty-five years or more are exempt from further payment, while retaining all the privileges of active members. Honorary members are not subject to dues.

ARTICLE XII.

UNPAID DUES.

All questions of indebtedness of members of the Society shall be referred to the Trustees, with power to act, or to report to the Society for its action. Any member failing to pay his dues after a period of one year and three months from the beginning of the fiscal year, without further notice, ceases to be a member of the Society; but upon payment of his indebtedness, he shall be reinstated without further formality.

ARTICLE XIII.

DISCIPLINE.

All questions of Ethics shall be adjudged in accordance with the Code of Ethics of the American Medical Association, and that of the Medical Society of the State of New York. There shall be three degrees of discipline, viz.; *Admonition, Suspension, and Expulsion.* A charge against a member for violating either of the aforesaid Codes of Ethics shall be made in writing, signed by the member making the charge, inclosed, sealed, and simply indorsed "Charge against a member," handed to the President, and by him submitted to the Committee on Admission and Ethics. The Committee shall furnish any member of the Society against whom a charge may have been preferred with a copy of the same, notifying said member and his accuser to appear before them, take testimony, and hear the defence.

Whenever the Committee on Admission and Ethics is prepared to report upon a charge against a member, the Secretary shall be required by them to notify the Society to that effect.

For *admonition,* a majority vote of the members present shall be sufficient; for *suspension,* a two-thirds vote of members present shall be necessary; for *expulsion,* a three-fourths vote of all members present shall be requisite.

ARTICLE XIV.

PRESENTATION OF SPECIMENS.

1. Members shall be called upon according to the order in which their names appear upon a register made on the evening of each meeting.

2. Members shall be allowed ten minutes for presentation of specimens, and this time shall not be extended, except by vote of the Society.

3. Every member shall be allowed five minutes for remarks on each specimen, and this time shall not be extended, except by a vote of the Society.

4. In discussions on specimens, members shall not be allowed to speak twice until all the members present have had an opportunity of taking part. A member shall not speak a third time, except by a special vote of the Society. This limitation shall not apply to mere questions and answers in the course of debate.

ARTICLE XV.

PRESENTATION BY CARD.

Specimens may be presented by card, and accompanied by a description. Their mode of presentation shall be left to the discretion of the President.

ARTICLE XVI.

ADJOURNMENT.

The Society shall adjourn at 10:30 P.M. There shall be no extension of the time, except by a special vote.

ARTICLE XVII.

AMENDMENTS.

These By-Laws shall take effect immediately. They may, by unanimous consent, be suspended at any meeting, but amendments shall be subject to the same requirements as those prescribed for amendments to the Constitution.

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DR. HANS ZINSSER, *President*

THE HISTOPATHOLOGY OF CALCIFICATION OF THE SPINATUS TENDONS, AS ASSOCIATED WITH SUBACROMIAL BURSITIS

ELI MOSCHCOWITZ, M.D.

The lesion is a common one. It is a frequent cause of shoulder disability and, as such, it has long been and still often is mistaken for brachial neuritis, rheumatism, etc.

In this affection the shoulder is stiff and painful, the symptoms and the lesion itself developing soon, as a rule, after trauma. Neither the pain nor the stiffness is characteristic in extent or incidence. The pain may be continuous or intermittent, spontaneous or on motion referred to the shoulder itself, or, more often, to the outer or anterior aspect of the arm. It sometimes radiates into the forearm or the neck. The stiffness may be manifested by awkwardness or by pain on motion. There is usually limitation of abduction, sometimes moderate, sometimes extreme. There is usually also limitation of rotation, internal, external or both. There is often tenderness in the upper deltoid region.

X-ray photographs of the cases studied showed well-defined areas of calcification within the spinatus tendons. These masses vary in size from a pea to a flattened nodule measuring 3–4 cm. in length. The masses may be single or multiple. The shadows are situated on the outer side of the tuberosity or head of the humerus, extending to beneath the acromion process.

PATHOLOGICAL EXAMINATION

The material studied consisted of tissue removed from three cases by Dr. Brickner.

Case 1. A portion of the supraspinatus tendon removed sixteen days after injury. Section shows a crescentic mass of tissue. Covering the border is a fairly well organized granulation tissue. Within this zone and filling the entire cavity of the crescent is richly cellular early granulation tissue consisting of many round cells and plasma cells. There are a large number of newly formed cells and the remains of a recent hemorrhage. Filling the cavity, of which this section is an arc, is a mass of amorphous necrotic tissue in which are discrete deposits of lime.

Summary.—This section reveals substantially two stages of granulation tissue; an outer, the older, and an inner more recent variety. These inclose a necrotic focus which has partly undergone calcification.

Case 2. Removed four months after injury. Due to imperfect orientation two separate masses are seen in the section. The first and larger consists of tendon fibers that have undergone hyaline degeneration in parts and in other parts calcification. From one aspect of the tendon there has sprung a large mass of granulation tissue, which is richly cellular, almost sarcomatous in appearance, consisting of large spindle shaped connective tissue cells with little fibrillar intercellular substance. In this tissue there are numerous newly formed young blood vessels. There is abundant lime within this granulation tissue in the form of sharply circumscribed discrete masses; some of the masses are in immediate relation to giant cells.

The second and smaller mass consists of hyaline tendinous tissue which has undergone almost complete calcification.

Summary.—A tendon that has undergone extensive hyaline degeneration and calcification, with manifest necrosis and secondary calcification of the tendon. In addition there is recent granulation tissue, in which are imbedded numerous calcified granules.

CASE 3. Removed one and a half years after injury. The tendon shows essentially a mass of tendon tissue enclosing a cavity containing necrotic detritus. Between the tendon bundles there is abundant firmly organized granulation tissue. While many of the tendon bundles have undergone hyaline degeneration the necrotic tissue either is loosely within the cavity or is directly continuous with the surrounding tendinous area. No calcification is discoverable, although Dr. Brickner assured the writer that abundant gritty masses were found at the operation; the lime must have been shaken out of the specimen during transport.

Summary.—A tendon that has undergone partial hyaline degeneration and is infiltrated with abundant connective tissue. Necrosis of tendon.

DISCUSSION

These specimens exemplify three progressive stages of a lesion that may be termed a "tendinitis."

The first common factor is the formation of a new granulation that corresponds, according to morphological criteria, to the duration of the illness.

A second common factor is the presence of necrotic foci, which in two of our three cases were demonstrably calcified, and in the third probably so. The explanation of the necrosis is obvious. The blood supply of a tendon is notoriously meager so that only a slight tear or injury is necessary to completely cut off the blood supply.

The pathogenesis of calcification within tendons is governed by the same conditions that apply to abnormal calcifications in other parts of the body. It has been definitely established that calcification occurs only in dead or inert tissues. It has thus a peculiar predilection for tissues that are necrosed or that have undergone hyaline degeneration.

The early calcification that has been noted in these cases (Dr. Brickner has seen cases in which X-ray shadows were demonstrable three days after injury) is remarkable, and can only be explained by the observation of certain experiments upon pathological calcification in other tissue of the body. For instance, Litten and Lick found lime in necrotic kidneys one day after ligation of the renal artery. Especially significant are the observations of Schujeninoff, who found calcification of muscle fibers 36 hours after suture of the divided muscle. Numerous experimental observations upon the healing and regeneration of tendons have been made, but these fail to throw any light upon the problem of calcification of tendons. This may be due to the fact that nearly all the observations were made upon the Tendo Achilles which may prove to be structurally different from the spinatus tendons. At all events, I am not aware of any disease of other tendons analogous clinically to the condition under discussion. In other words, the associated symptom-complex, namely, trauma, necrosis and early calcification of a tendon, attended by protracted disability, is apparently nowhere observed except in the spinatus tendons.

The only conditions to which this malady may be likened are the comparatively uncommon traumatic "ossifications" discoverable by X-ray in various tendons, especially around joints. To these may be added the "calcaneus sporn" arising from the posterior surface of the os calcis and extending into the Tendo Achilles; "Rider's bone" or "ossification" of the adductors of the thigh; and "Drill bone," or "ossification" at the insertion of the deltoid. Myositis ossificans is in all probability an allied condition. The possible objection that these shadows are bone and not lime does not entirely disqualify the parallelism. In the first place, pathological evidence that these shadows are bone and not lime is still wanting. In the second place, it is now generally conceded that pathological ossification cannot occur without premilinary calcification, so that these shadows in their early stages must have represented deposits of lime. These lesions, however, cannot be compared either in the matter of frequency or in the early development of the lesion.

The lesion described in the spinatus tendons has, however, certain precise pathological analogies. We refer to the rare multiple nodes described in tendons by Krause and Trappe, Neuwirth and L'Hermitte, which are embolic in origin and are probably due to calcification of an infected focus.

The gouty deposits in tendons are also identical pathologically, the only difference being that instead of lime the deposit consists of urates.

Discussion:

Dr. Brickner: Dr. Moschcowitz's presentation is especially interesting because it is the first systematic histologic study of calcareous deposits in the spinatus tendons and is based on the largest series of cases that have been studied surgically. I think we may say that we now have a clear notion of this variety of "shoulder disability" in its histologic and surgical aspects, but there remain many problems to be solved. Among these is the peculiar tendency of the supraspinatus, sometimes the infraspinatus tendon, to the early deposit, sometimes large and multiple deposits, of lime salts, after comparatively mild violence. I have seen twenty cases in the last couple of years.

I am not sure that Dr. Moschcowitz is justified in calling calcifying spinatus tendonitis the chief cause of shoulder disability, but I believe subacromial bursitis, which often has this tendon lesion associated with it, *is* the most common cause.

I use the term "shoulder disability" in preference to "stiff and painful shoulder" because either the pain or the stiffness may greatly preponderate or exist alone.

Codman first gave a very comprehensive description of subacromial bursitis, anatomically and clinically, and in two articles correctly located one of the varieties of deposit—the semi-fluid, extratendinous—as beneath the bursa, but he spoke of it as of a cyst, which is wrong, for there is no capsule or limiting membrane. Wrede, in the report of a single case, also located the deposit correctly, viz., in the supraspinatus tendon. Aside from these two, all writers on subacromial bursitis and tendon deposit have been in error. Indeed, the literature of this subject strikingly indicates the not uncommon tendency in medicine to jump at wrong conclusions, and the frequency with which fallacies are transmitted from one author to another.

Painter, who was the first to observe the calcareous deposit, reported that he evacuated it from within the bursa, although Codman, who was present at the operation, observed that it came from beneath the bursa. In two other cases operated upon by Painter he found no deposit (because he did not look beneath the bursa), and he assumed that the X-ray shadow was produced by thickening of the bursal walls. Since then all surgeons and radiographists, excepting Codman and Wrede, have regarded these shadows as due to thickenings of, effusions in, or calcifications of the bursa. Holzknecht, for example, calls the calcareous mass a "bursalith." Cumston recorded one of these cases as a suppurative bursitis, although there was none of the familiar symptoms or signs of suppuration, and in spite of the fact that the "thick, yellowish pus" evacuated "from the bursa" was not examined. When, as is often the case, the bursal walls are adherent, an incision into the sac, unless made with this consideration in mind, is apt to go through the floor of the bursa, evacuating from beneath the often cheesy deposit, which thus appears to come from within the bursa. Baer, who also operated upon cases of subacromial bursitis not only repeats the error that "replacement of the bursa by scar tissue causes a shadow in the radiogram," but also assumed that "if the process is a tuberculous one the cavity may be filled with a dense, cheesy material." I have seen but a single, doubtful case of tuberculous subacromial bursitis. I have had one of these "cheesy" calcareous deposits studied bacteriologically; it was not only germ-free (smears and cultures), but also produced no reaction in guinea-pigs.

Another fallacy has been the assumption of bacterial or toxic irritation as an etiology of subacromial bursitis. Such an assumption has never been substantiated. If the condition were bacterial or toxic we ought to see it also in the olecranon, trochanteric and other bursæ. Excluding the rare tuberculous and the occasionally suppurative (septic) variety, subacromial bursitis (the bursitis of shoulder disability) is traumatic in origin, due, I believe, to bruising the bursa and the tendon between the head of the humerus and the acromion process. The trauma may be either an external or an internal violence. As instance of an external violence, a fall upon the outstretched arm, which jams the humerus against the acromion. A physician whom I operated upon this week, thrown by an automobile *upon* his shoulder (he says) seventeen days previously, had dense adhesions in the bursa and two discrete, large, dry, powdery calcareous deposits in his supraspinatus tendon. Very often there is no history of injury. In these cases the lesion results from mild internal violence, viz., a sudden or unduly vigorous abduction of the arm, as in the lurch of the body while hanging to a street-car strap, exercising on flying rings, leaning on the outstretched arm as in scrubbing the floor, etc. In all these forms of injury the bursa and tendon (supraspinatus, infraspinatus, or both) are compressed between the two bony surfaces. There results, as may be seen at operation, a hemorrhagic infiltration of these structures and, often, a small tear of the supraspinatus near its insertion, sometimes through its entire thickness. There is no doubt that a tear of this tendon can be produced by sudden abduction, in the adult. Codman reported three, and operated upon two, cases of complete rupture of this tendon. In one, a woman, it resulted from throwing a heavy blanket over a clothesline; in the other, a man, from tightening a saddle-girth. In both cases something was felt to snap, and the arm fell limp.

There are two forms of the calcareous deposit, in my experience,—one chiefly extratendinous, and associated with a tear in the tendon; the other, single or multiple, entirely within the tendon and with no surface indication of tendon injury. Either form, independent of the acuteness or chronicity of the case, may be semi-fluid or dry and gritty. While Dr. Moschcowitz is no doubt correct, as a pathologist, in calling this a "calcification of the tendon," surgically it is to be regarded only as a calcareous deposit. There is no extension, grossly, of the calcification beyond the torn fibers immediately surrounding the deposit.

We have both referred to the early appearance of the lime. I have operated upon cases sixteen and seventeen days after the injury, and I have seen the shadow of the deposit ten and five days and even, if the history was reliable, one day after injury.

The operative plan I follow consists in splitting the deltoid muscle, carefully incising the bursal roof, dividing all bursal adhesions, incising and dissecting up the floor of the bursa, removing the deposit from the surface or from within the tendon (usually the supraspinatus), trimming the edges of the tendon tear or incision, suturing the tendon, and then suturing, successively, the floor of the bursa, its roof, the muscle, and the skin, without drainage. I smear a very thin layer of vaseline in the bursa. The arm is abducted in plaster for about eight days.

GRAM-NEGATIVE MICROCOCCUS CAUSING FATAL ENDOCARDITIS

CALVIN B. COULTER, M.D.

(Pathological Laboratory, Presbyterian Hospital)

The presence in the blood stream of the gonococcus and meningococcus as a general bacteriæmia with septic manifestations has been observed repeatedly. In the case to be reported here, there was presented the familiar clinical picture of infective endocarditis with the recovery from the blood during life, and from the organs at autopsy, of a Gram-negative micrococcus which appears to be closely related to the gonococcus, but differs from it in important particulars, and represents probably a new species.

The patient, a Swiss cabinet-maker of 45 years, entered the Presbyterian Hospital November 6, 1914, suffering with dyspnœa and general weakness and repeated vomiting. The onset of symptoms had occurred three months previous with chill, followed by progressive weakness and night sweats. The patient presented the signs of cardiac enlargement with a loud systolic murmur, and pulse of auricular fibrillation. The liver and spleen were enlarged. No petechiæ were found. Diagnosis of malignant endocarditis was made.

Blood Examination, Nov. 7.—Leucocytes, 8100. Polys., 69 per cent.; Monos., 28 per cent.; Eosin., 3 per cent. Blood culture, positive.

Disease ran an irregular febrile course, temperature reaching 103°–4°. There were recurrent attacks of tenderness over the spleen and associated with this on November 17 there was swelling and redness over the 5th left metatarso-tarsal articulation, and on December 2 swelling and redness appeared over right 1st metacarpo-carpal joint, with tenderness of pulp space of adjacent forefinger.

November 17.—Gonococcus complement fixation serum, anticomplementary.

November 27.—Leucocytes 12,200. Polys., 79 per cent.; Lymphos., 29 per cent.

December 7.—Leucocytes, 11,400. Polys., 81 per cent.; Lymphos., 18 per cent.; Eosino., 1 per cent.

Blood cultures were positive on November 7, November 11, November 13, November 27, November 30 and December 16. Last culture plates showed 6 colonies per c.c.

December 25.—The patient developed rather suddenly a left hemiplegia and died after about 36 hrs. with signs of cerebral hemorrhage.

At *autopsy,* 8 hrs. post mortem, was found an early bronchopneumonia of both lungs. The heart was large, weighing 520 grams, and showed hypertrophy and dilatation. The valves were normal, except for the mitral which showed on the margin of its posterior cusp a rounded and thickened tag of tissue, just large enough to contain a circular ulcer 6 mm. in diameter. This ulcer completely perforated the valve; its margins were firm and raised, its cavity filled with fibrin; in one spot it showed a minute vegetation. This small patch was the only evidence of vegetative endocarditis throughout the heart.

In the wall of the jejunum was found a small thrombotic nodule, 8 mm. in diameter. The liver and kidneys showed a state of chronic passive congestion. The pelvis of the right kidney contained pus. In several of the kidney glomeruli were seen in sections necrosis of a portion of a glomerular tuft, and later stages, with fibrosis and adhesions to the capsule, of the lesion considered characteristic of *Streptococcus viridans* endocarditis.

On a branch of the right middle cerebral artery was found an embolic aneurysm 7 mm. in diameter. Rupture of this had caused an extensive hemorrhage into the brain tissue, and had been the immediate cause of death.

Cultures at Autopsy.—Heart's blood: sterile. Kidney: sterile. Pyelitis: sterile. Lung: streptococcus and pneumococcus mucosus. Hemorrhage in brain: same organisms as found in lung. Spleen and the embolus in the jejunum: pure culture of Gram-negative micrococcus. Section through the ulcer on the mitral valve shows clumps of small cocci lying in the zone of fibrin just beyond the necrotic valve margin, and reaching the surface of the ulcerated area in one spot.

DESCRIPTION OF ORGANISM

The microorganism isolated appears as a very small coccus, in size equal to the smallest forms seen in the ordinary *Staphylococcus aureus*. There is hardly any visible variation in size between individuals in a given culture and in growths on the various media. Diplococcus forms are frequent and resemble in form the biscuit-shape of the gonococcus. Small clumps of three to eight are most numerous. Curious bacillary forms occur, the shorter of which can be resolved into a chain of four or five cocci, the larger appear homogeneous, and reach twice that length.

Stained by Gram's method with the meningococcus and hay bacillus on the same slide as controls, this coccus is invariably decolorized. Methylene blue and safranin alone stain it poorly. Fuchsin gives a more intense stain.

The first isolation from the blood was obtained on 1 per cent. dextrose-agar plates, poured at the bedside, but growth did not occur in inoculations into dextrose-meat-infusion-bouillon. Attempts at isolation from the blood were made by other investigators, using media of the same formula, but were never successful. On blood plates the colonies appeared after 48 hours incubation at 37° C. Typical colonies were 0.5 mm. in diameter, dark green in color and surrounded by a diffuse zone of light olive green. On surface cultures the *characteristic delicate growth* is visible after 12–24 hours, and reaches its maximum at 48 hours. Colonies are always discrete, and macroscopically, are of two types: The smaller and more numerous are minute and almost invisible to the naked eye except when crowded together, where they give the appearance of a confluent growth. The larger colonies attain a diameter .25 mm., and appear as glistening transparent droplets. Microscopically, the colonies are alike, they are roughly spherical, dry, and appear made up of minute refractile granules. When a colony is touched with the needle it is easily dislodged, and moves about on the surface of the medium without breaking up.

Simple dextrose-agar was used successfully for the isolation from the intestinal embolus, and transplants in the second generation from the blood plates grew well in this medium. Growth did not occur at first on Loeffler's blood serum, but does so after artificial cultivation. Ascitic dextrose-agar is the most favorable medium, and after continued cultivation upon it the separate colonies are opaque, white, with wrinkled surface and outline, and tend to umbilication. They resemble minute bread crumbs. Under cultivation there is no increase in size of colonies. On the simple solid media no change has occurred, and growth appears as on first isolation.

In fluid media and in the water of condensation of solid media, growth is peculiar. After 8–12 hours discrete granular masses up to 0.5 mm. in diameter are noticed in the bottom of the flask, clinging to the sides of the glass. The supernatant fluid is perfectly clear. After 36 hours a general cloudiness begins and increases for 2–3 days. Old broth cultures show a delicate milk-white veil adhering tenaciously to the bottom and sides of the glass and a few delicate filaments springing from the veil float in the fluid.

Growth is as vigorous anaerobically as aerobically, and neither macroscopically nor microscopically do the two cultures differ.

Microscopically this organism appears like a minute gonococcus or meningococcus, but large involuted forms, metachromatic staining, and rapid autolysis, do not occur. The isolation upon serum-free media, and the vigorous growth throughout bouillon tubes, are important points of differentiation from the gonococcus. The firm dry colonies and spontaneous sedimentation in broth cultures make the organism resemble the *Micrococcus pharyngis siccus,* but the extreme delicacy of growth of this unclassified coccus is unlike that of any of the Gram-negative micrococci with which I am familiar.

Dextrose is the only sugar fermented. In litmus milk there is a slight change in color, but levulose and galactose used alone are not acidified. Thus the fermentation reactions suggest the gonococcus.

An immune serum was produced by intravenous injections of killed cocci into rabbits. This agglutinated the coccus in a dilution of 1 : 32 and very slightly in 1 : 64. This serum was tested against a strain of meningococcus with entirely negative results. A strain of gonococcus was agglutinated in 1 : 32 dilution. Portions of serum were then absorbed with the homologous coccus and the gonococcus. Serum absorbed with its specific coccus no longer agglutinated the gonococcus while after absorption with the gonococcus, it still agglutinated the specific coccus in dilution of 1 : 16.

The coccus was not agglutinated by the patient's serum, nor by a commercial antigonococcus serum, which however gave negative results with the gonococcus strain tested above, nor with a meningococcus immune serum.

This coccus is highly pathogenic for mice. One fifth of the 48-hr. growth on a blood agar slant killed a white mouse in 4 hours. The coccus was regularly recovered from the heart's blood of experimentally injected mice.

Guinea-pigs succumb in 12 hours to large doses intraperitoneally; chronic infections have not been set up, using smaller amounts of material for inoculation.

Similar results have been obtained with rabbits; small doses do not set up a chronic infection; large doses intravenously result in death in 60 to 72 hours. The organism has not been regained from the blood during life of experimental rabbits, nor from the heart's blood at autopsy, and it seems probable that death in guinea pigs and rabbits is due to the liberation of a toxin from the bodies of the cocci.

Attempts have been made to obtain a soluble toxin, by passing bouillon cultures, grown from periods varying from 48 hours to 10 days, through a Berkefeld filter. The filtrates have been uniformly non-toxic for white mice and guinea-pigs.

CONCLUSIONS

Morphologically, this organism seems related to the Gonococcus-Meningococcus group. Culturally, it does not conform with any organism with which we are familiar, although here too there are suggestive points of resemblance to the well-known Gram-negative diplococci. Accurate differentiation on the basis of serum reactions can not be made until an immune serum has been developed with an agglutination titer higher than 1 : 64, the maximum so far obtained. Preparation of such a serum has been rendered difficult by the toxicity of the coccus for rabbits.

CASE OF SUB-LEUKÆMIC LYMPHADENOSIS

MORTIMER WARREN, M.D.

(Pathological Laboratory, Roosevelt Hospital)

The title given to this case report is used more in a descriptive than in a diagnostic sense in an attempt to define the pathological conditions found.

In order to arrive at any kind of a diagnosis it is, of course, essential to know as much as possible of the clinical history and course of the patient, especially since the objective evidence is limited to the histological appearance of one lymph node and to the blood findings. I therefore will give briefly the history and status of the patient.

Clinical History.—A white male of twenty-six years, born and brought up in the British West Indies,—a railroad clerk by trade,—entered The Roosevelt Hospital on June 5, 1914, service of Dr. Evan M. Evans, History No. D–6192. His chief complaint was weakness, gradually increasing for the past fifteen months; at times pallor and yellow color had been noted by his friends. For a week before admission he had some evening fever and slight sweats with chilly sensations. Past history is unimportant aside from that of malaria when in the British West Indies, with frequent dosage of quinine; he had, however, no chills.

Examination.—A young man of 116 pounds, with evident pallor and slight icterus. Moderate enlargement of axillary, inguinal and epitrochlear lymph nodes. Spleen edge is palpable 2¾ inches below costal margin. Liver edge not felt. Heart and lungs negative. No sore throat. There was a petechial spot in the soft palate which subsequently disappeared.

During his stay in the hospital from June 5 to July 3, 1914, examinations of urine were negative, aside from the presence of bile in one instance, and of urobilin in another. The gastric contents showed lack of free hydrochloric acid and the presence of occult blood. Fæces were negative for ova and parasites. Temperature was irregular ranging from 99° to 102°, but towards the end of his stay lessened in height. A blood culture was negative. Wassermann was negative.

On June 10, 1914, a pectoral lymph node was excised, section of which (B–7028) I wish to show and present for your opinion and diagnosis.

The *blood findings* were interesting, and were, I believe, directly related to the changes in the lymphatic apparatus as witnessed by the histological structure of the excised lymph node. Hæmoglobin, 54; leucocytes, 18,000; erythrocytes, 4,400,000. Polynucleus, 42.6 per cent. Lymphocytes, 12 ordinary lymphocytes; 19 stimulation forms; 18.6 pale cells. Transitional, 1. Eosinophiles 5.3. Mast cell, 1.3.

DISCUSSION

The leucocyte count was sub-leukæmic, being 10,000 to 20,000 with some forty-odd per cent. of mononuclears belonging to the lymphocytic series, with a constant increased per cent. of eosinophiles.

Anæmia was present at all times, more marked in the individual red blood cells than in the total count: of chlorotic type, with considerable pallor and variation in size, shape and staining reaction of the cells. Occasional normoblasts were seen. Platelets in smears were evidently increased.

At first we tried to make the leucocyte formula fit into the myeloid series but the individual mononuclears, though deeply basic in staining reaction of the protoplasm, were scanty in nucleoli and were non-granular. They gave no oxydase reaction and the blood was practically free from myelocytes or any cells which could be considered transitional forms in the neutrophilic group, though transitionals of the usual variety were present in fair number. The lymphocytes could be divided into three groups: (1) The ordinary small cell with its little protoplasm. (2) Large pale cells with azure granules,—likewise normal. (3) Abnormal deep, basic cells of the type of "stimulation" forms. Smears of the gland showed all the types of cells seen in the blood beside larger endothelioid cells.

Examination of sections led to a variety of diagnoses. I was inclined to some form of metaplasia at first,—later to the diagnosis of "Hodgkin's," a provisional diagnosis of which Dr. Mann had already made. This diagnosis was strengthened by the result of a growth from a portion of the node on egg media, which proved to be *Bacillus Hodgkinii.* Further study has, however, convinced both Dr. Mann and myself, as well as Dr. Cunningham, who has recently reviewed all our cases of Hodgkin's disease—some twenty in number—that this is not a proper diagnosis. Here I might say that Dr. Butterfield examined the smears of blood and sections of the gland and did not consider that it conformed to Dorothy Reed's description of Hodgkin's disease, but was unwilling to completely correlate the blood and tissue findings. A section of the gland, a smear of the blood and a plant of the culture were sent to Dr. Bunting in Wisconsin, who reported both the gland and organism typical of Hodgkin's disease. He regarded the blood differential as rather higher in lymphocytes than usual, except in early cases of the disease.

The gland itself shows a varying fibrosis which is marked only in portions and is somewhat whirling in character. The follicles as such are not evident; there are, however, focal collections of large mononuclears showing active mitotic division,—areas which may represent the germinal centers and the cells of which may be germinal center cells. Similar cells are also scattered throughout the gland in the interstitial tissue, and are likewise seen in the capillaries and in the lymph spaces. These cells appear to me to be the source of the abnormal cells, the lymphocytic stimulation cells of the blood. There are larger cells with well marked nucleoli occurring singly in the interstitial tissue, and in areas there are pale staining, endothelioid phagocytic cells, but there are no multinucleated giant cells, or giant cells with lobed nuclei. The preponderating cell is a lymphocyte of the small type. Occasional mast cells and scattered eosinophiles are present. I think I can see all sorts of transition stages between the large, actively dividing cell and the small lymphocyte, in certain examples forming a characteristic plasma cell.

I believe this is a case of leukæmia of atypical form: It is evidently not acute—patient was heard from about two months ago and was then in fair condition—and it is not of the chronic type, unless in an early stage. A count made one week after leaving the hospital showed 55,000 leucocytes.

One can readily believe the anæmia to be secondary, possibly in part hæmolytic in character, thus accounting for the icterus and the urobilinuria, though there were no definite signs of active regeneration. It is, of course, impossible to draw any conclusions as to the state of the bone marrow.

Discussion:

DR. ZINSSER: I would like to ask Dr. Warren's views on the possible relation of this case to cases in which *B. Hodgkinii* is found.

Dr. Warren: We found this organism in a mediastinal sarcoma and in two or three tubercular lymph nodes. This one grew in pure culture in egg media.

Dr. Coulter: I have cultivated a similar organism in Hodgkin's disease from the cervical lymph nodes, and in another case similar to this one of Dr. Warren's, from excised lymph nodes.

Dr. Taylor: Doctor Pease has been making cultures at our hospital from various cases of glandular enlargement and has isolated by means of shake-cultures of glucose-ascitic-agar diphtheroid bacilli. These have been found in a number of cases of typical Hodgkin's and also in what was histologically lympho-sarcoma and a case which at autopsy was apparently tuberculosis. The possibility of a combination with Hodgkin's disease in this case could scarcely be eliminated, but it seemed to us that the whole process might be explained on a tubercular basis.

Dr. Warren: We have had frequent contaminations of blood cultures of a diphtheroid organism. Whether it is related to *B. Hodgkinii* I don't know.

Dr. Garside: In erysipelas I have taken the skin and rubbed it on the plate and got a pure culture of streptococcus. Dr. Bunting attempted to reproduce the disease, did he succeed?

Dr. Warren: He produced lymphatic enlargement and necrosis, but I don't think it is the same as in human beings.

Dr. Coulter: Did the case run a febrile course?

Dr. Warren: 99 to 102.

ADENO-CARCINOMA OF UTERUS WITH METASTASES IN BOTH TUBES

L. W. STRONG

(*Woman's Hospital*)

I have an interesting case of adeno-carcinoma of the body of the uterus with metastases in both tubes. So far as I am aware there is no case in the literature of a double metastasis in the tubes. There are, of course, cases of cervical carcinoma with general metastasis, and there are cases of carcinoma spreading to the tubes by direct extension, but this case shows no connection between the original tumor and the metastases.

The point of greatest interest in the case is the method of occurrence of the metastases, whether by the blood or lymph stream or by creeping up of the cells from below, or possibly by a multicentric origin of the tumor. There is no evidence of extension by the lymph or blood stream in this case, nor is it easy to imagine that if that were the method of origin, the metastases should occur in the epithelium of the tubal mucosa without previous involvement of some lymphatic structure.

The slides bring out two points bearing on this question. The first is that there is a general inflammatory reaction in the entire genital mucosa which suggests the possibility that a chemical or other irritant may have caused a multicentric development. The second point is that the lumina of the fundal glands and tubal mucosa show groups of very large desquamated cells which look like carcinoma cells, and it is possible that these represent wandering cells which have embedded themselves higher up in the tubal mucosa. The cervical glands of this case show a picture which might well represent appositional growth, since there are glands one half of which present perfectly regular epithelium, while on the other side of the lumen the cells have every appearance of the carcinoma which is directly adjacent to them. This idea of appositional transformation has been generally discarded for the unicentric idea of carcinomatous growth. Schöttlander still holds to the appositional transformation. Ribbert, Borst and others hold to the unicentric origin, while v. Hansemann and Lubarsch think that both methods of origin are possible.

If carcinoma is always a definite entity there can be no such thing as appositional growth, that is, a transformation of cells at the border into carcinoma cells; but, the general belief is that carcinoma is not such a fixed entity, as indicated by the transitions between carcinoma and benign growths, as adenoma, and also by the transitions between carcinoma and the so-called precancerous changes, so often seen in the breast. It is these unexplained appearances which are the support of the appositional transformation idea.

AN INTRACUTANEOUS METHOD FOR TESTING THE VIRULENCE OF DIPHTHERIA BACILLI

ABRAHAM ZINGHER, M.D., AND DAVID SOLETSKY, B.S.

(From the Research Laboratory, Department of Health, New York City)

The testing of the virulence of isolated strains of diphtheria and diphtheria-like bacilli is important for a number of reasons, the following being probably the most important:

1. It has been found that 4–8 per cent. of individuals in localities where diphtheria is endemic are carriers of bacilli morphologically and culturally like the diphtheria bacillus, but non-virulent in 30–50 per cent. of the cases. At the Willard Parker Hospital, Wilcox and Taylor found that 4.5 per cent. of the cases admitted to the scarlet fever wards were bacillus carriers, and of the isolated organisms only one half were virulent.
2. According to Neisser individuals who become persistent carriers after an attack of diphtheria show only non-virulent forms in fully 20 per cent. of cases. Such individuals could be discharged from quarantine if this fact were established.

Morphologically, the non-virulent strains cannot be separated from the virulent, while in their sugar fermentation reactions the two types are in a majority of instances similar. Hence, the animal test must be employed for final diagnosis in doubtful cases. The method so far in vogue for testing the virulence of diphtheria bacilli has been to isolate the strain, grow it for 48 hours in ascitic broth (1 part ascitic fluid + 2 parts veal broth), and inject 1 c.c. of the broth culture subcutaneously into a guinea-pig. A control pig is injected with the same amount of the broth culture, and also a small quantity of antitoxin (0.5 c.c. of a 100 or 200-unit antitoxin, which cannot be used for other purposes). If the strain is a true diphtheria organism, the control pig will live, while the test pig will die in 2–3 days, and the autopsy will show the typical lesions of death from the effects of diphtheria toxin; that is, subcutaneous œdema at the site of injection, often extensive, congested and hemorrhagic adrenals, fluid in the pleural cavities, congestion of the lungs with areas of partial consolidation.

This method is reliable, but an autopsy should be performed in all cases, in order to exclude death from other causes.

Recently M. Neisser has suggested that the virulence of cultures be tested in the following way: One loopful of a 24-hour Loeffler slant of the organism is suspended in 1 cc., 10 c.c., and 100 c.c. of physiological salt solution, and 0.1 c.c. of each suspension is injected intracutaneously on the abdominal surface of a guinea-pig. As a control some antitoxin containing 8 units per c.c. is added to an equal volume of the heaviest suspension, and 0.1 c.c. of the mixture is injected intracutaneously in the same guinea-pig. True virulent diphtheria bacilli will cause a local inflammatory lesion, with superficial necrosis, in 48–72 hours, the intensity of the reaction depending upon the number of injected organisms and their virulence. The skin at the site of the control injection should remain normal in appearance.

This method is analogous to that of Römer for the determination of small amounts of diphtheria antitoxin in sera. This consists of the intracutaneous injection of varying mixtures of the unknown antitoxic serum and a standard toxin; a slight excess of toxin produces a local necrosing lesion, while a neutral or over-neutralized mixture shows no effect on the tissues at the site of injection.

The method recommended by Neisser is fairly satisfactory, but, following his directions, we have occasionally noted that the direct addition of antitoxin to the bacteria in the control injection immunized the animals sufficiently to affect the test lesions to a considerable degree. If the amount of antitoxin added is diminished to avoid this general immunization, the local action of the bacteria is not completely inhibited, and lesions are found in both test and control areas.

For this reason the following modification of Neisser's method which has been found to be both reliable and economical is suggested.

Method.—Two guinea-pigs are used for the testing of from

4 to 6 different strains. One pig serves as a control and receives 0.5 c.c. of antitoxin (about 200 units per c.c.) intracardially at the time of making the tests, or intraperitoneally 24 hours before. The intracardial injection is the better, as it produces a complete inhibition of the local action of virulent bacteria in the control injections. The hair on the abdominal surface of each pig is removed, either by the application of a paste made of barium hydrosulphide, or preferably by simply pulling the hair out. This can be easily done, with less pain probably than is associated with the prolonged irritation following at times the application of the depilatory.

For the bacterial emulsion, a fresh 24 hour growth from an ordinary Loeffler slant is suspended in 25 to 30 c.c. of normal saline. It is important that the growth be not more than 24 hours old, since many of the bacteria die if the culture is kept for 48 hours or longer in the thermostat. Ice-box preservation of grown cultures also kills many of the organisms. Loeffler slants are used similar to those furnished by the New York City Department of Health for the purpose of diphtheria diagnosis. They should have a fairly uniform surface size and be sealed with paraffin to prevent drying of the medium. It is also important that the medium be not too acid, since excess of acid is apt to inhibit growth to a considerable degree.

Suspensions of the cultures to be tested are prepared in the above way, and 0.1 c.c. of each is injected intracutaneously into both pigs. For the purposes of injection the abdominal surface is divided into 4 to 6 areas, depending upon the size of the guinea-pig, and the injections are made as far apart as possible in order to avoid a fusion of the lesions. Four strains can be tested out on a medium-sized guinea-pig, and six on a larger one, without the danger of overlapping. A .5 c.c. or 1 c.c. Record syringe with a very fine steel (Burroughs Wellcome & Co. No. 3) or platinum iridium needle (Burroughs Wellcome Co. No. 22) is suitable for the injection. If the injections have been made properly a circumscribed elevation appears, which persists for 1 to 2 minutes.

The results of the tests are noted in 24 to 48 hours. Virulent strains produce a definite local inflammatory lesion, which shows a superficial necrosis in 48–72 hours. In the control pig the skin remains normal, when the injections are accurately carried out, With non-virulent strains, no lesion will be found in either control or test animal.

In this way, four cultures can be tested on two animals, as compared with eight animals necessary in the older way. By the use of large pigs, on whom six tests can be made, 10 out of 12 pigs can be saved, an advantage which is considerable when a large number of strains are to be tested.

We have tested by this method 20 non-virulent and 40 virulent strains of diphtheria bacilli, and the results obtained corresponded exactly with those obtained with the subcutaneous test for virulence.

A little practise is required for the proper performance of the injection; but with good technique, fresh Loeffler slants, and a proper 24-hour surface growth, the results will be found to correspond exactly with those obtained in the usual and less economical way for determining the virulence of diphtheria and diphtheria-like organisms.

We wish to thank Dr. Wm. H. Park, Director of the Research Laboratory, for many helpful suggestions in carrying on the work.

Discussion:

DR. HOPKINS: I would like to ask if necrosis could be produced by any other organism of this group, and were tests made to determine the specific action of the organism by using antitoxin as control.

DR. ZINGHER: The local action of the diphtheria bacillus and of its toxin is very characteristic, and no lesions were produced when the *Bac. Xerosis* or *Hoffmanni* and about twenty non-virulent strains of diphtheria-like bacilli were tested by the intracutaneous test on the pig. The action of antitoxin injected intracardially into the control animal was found absolutely specific in counteracting the action of the virulent diphtheria bacilli.

DR. LAMBERT: When the animals die, are the lesions in the adrenals absolutely characteristic; can they be mistaken for anything else?

DR. ZINGHER: The congested and hemorrhagic adrenals in the presence of the other typical lesions are considered characteristic of the action of the soluble toxin of the diphtheria bacillus.

DR. WARREN: What do you do with the guinea-pigs afterwards?

Dr. Zingher: We keep them for one or two months and then use them for complement.

Dr. Johnson: Was there any fluid around the pericardium?

Dr. Zingher: In the test animals, the total amount of bacteria injected represents as a rule, a sublethal dose. We have found occasionally in the regular test of virulence a small amount of fluid in the pericardium.

Dr. Johnson: Did you test the blood and pleural fluid to find the organism?

Dr. Zingher: We have found bacilli once or twice in several examinations of the heart's blood post-mortem. According to Wright 5 per cent. of the animals show the presence of bacilli in the heart's blood. The pleural fluid was not tested.

Dr. Zinsser: I am surprised at the large number of non-virulent strains. I ran through eighty and found only a few non-virulent. They all fermented like true diphtheria. Is the absence of virulence in carriers only temporary, has this been tested, and can these strains be brought to virulence?

Dr. Zingher: Among scarlet fever patients admitted during the past year to the Willard Parker Hospital, Wilcox and Taylor found that about one half of carriers showed non-virulent organisms. In those who became carriers in the wards after admission fully 95 per cent. of the strains were found virulent. The absence of virulence in the organisms in the carriers harboring non-virulent bacilli is probably permanent. Roux and Yersin are of the opinion that both types are present in cases of diphtheria, the preponderance of each strain, however, varying with the severity of the disease; but that during convalescence all virulent strains undergo a definite diminution in virulence. Graham-Smith, Loeffler, and Park and Williams believe that the individual virulence or non-virulence is permanent with the strain. Dr. Williams following the methods advocated by several who claim they made this transformation failed to obtain the change. We are attempting at present to change a non-virulent into a virulent strain, according to the method suggested by Thiele and Embleton for the *Bac. Hoffmani.* They claim to have changed such a strain to a virulent diphtheria bacillus.

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DR. HANS ZINSSER, *President*

AGGLUTINATION OF TREPONEMA PALLIDUM

HANS ZINSSER, M.D., AND J. G. HOPKINS, M.D.

We wish to report some recent experiments which demonstrate definitely the presence of immune bodies in animals immunized with cultures of treponema pallidum. The earliest attempts to demonstrate antibodies in syphilis were in general unsuccessful, Failure to obtain immune reactions was due doubtless in a large part to the fact that the earlier workers did not have cultures of the organisms to work with and that the suspensions of organisms obtained from syphilitic lesions were too poor in treponemata to make success possible.

Noguchi in 1912 obtained positive complement fixation reactions in the sera of patients with advanced syphilis but not in those of inoculated rabbits, when he used as antigen aqueous extracts of syphilis cultures or extracts obtained from rabbit lesions. Kolmer, Williams, and Laughbaugh soon afterward reported similar results, using extracts of concentrated cultures. Positive results were given by a considerable number of sera from cases of human syphilis, but much stronger reactions with the sera of rabbits treated with treponema cultures.

In the course of our work with the treponema we have immunized a number of rabbits with suspensions of the organism obtained by centrifuging fluid cultures, and in this way have obtained suspensions containing enormous numbers of treponemata. The suspensions before injection were killed by heating one half hour at 56° C. In testing the sera of these immunized animals against similar unheated treponema suspensions we have observed powerful agglutination, which was observable both macroscopically and under the dark field, one of our rabbits giving very marked agglutination in a dilution of 1:2000. We have observed the agglutination, both when using a suspension of our own Strain "A" against which the animals were immunized, and also with a suspension of N-1, a strain isolated by Dr. Noguchi. The sera of some normal rabbits also showed power to agglutinate the treponema pallidum, but we have not so far obtained this reaction in a dilution greater than 1:20.

EXPERIMENT I. (MACROSCOPIC)

Suspension Strain "A."

Serum Rabbit "609" (intravenously treated with five injections of "A" suspension ranging from 1 c.c. to 5 c.c.).

Each tube contained 0.5 c.c. of serum dilution and 0.25 c.c. of suspension.

	Serum 609. First Bleeding		Serum 609. Second Bleeding		Normal Rabbit Serum	
	2 hrs.	15 hrs.	2 hrs.	15 hrs.	2 hrs.	15 hrs.
Conc.	+++	+++	+++	+++	+++	+++
1:2	+++	+++	+++	+++	+++	+++
1:5	+++	+++	+++	+++	+++	+++
1:10	+++	+++	+++	+++	+++	+++
1:20	+++	+++	+++	+++	±	±
1:50	+++	+++	+++	+++	0	0
1:100	+++	+++	+++	+++	0	0
1:200	+++	+++	+++	+++	0	0
1:500	++	+++	+++	+++	0	0
1:1,000	±	0	Not		0	0
1:2,000	0	0	Set		0	0
1:4,000	0	0	Up		0	0
Salt Sol.	0	0	0	0		

We have not yet proceeded far enough with our experiments to determine whether these reactions can be obtained with the sera of syphilitic patients or with those of rabbits showing syphilitic lesions.

We submit the preceding protocol as an example of the macroscopic tests made with the sera of immunized and of normal animals.

A CASE OF MULTIPLE MYELOMA

W. G. MACCALLUM, M.D.

Clinical History.—B. C., a woman aged 53, was admitted to the Presbyterian Hospital December 5, 1915, complaining of stiffness of neck and shoulders. This appeared gradually during the past three months but was not associated with any pain. On admission neck was held very stiffly, apparently entirely on account of spinal rigidity, as the muscles were not tense. There was a deep groove in the ribs running parallel with the sternum just outside the costo-sternal junction. The ribs were easily moved or bent and on very slight pressure gave the impression of being crushed. Thoracic and abdominal organs showed nothing abnormal. The spine was held rigid throughout. Both femora and tibiæ showed outward and forward bowing. No tenderness anywhere.

Blood count, examination.—

Leucocytes	9,000
Polymorphonuclears	54 per cent.
Lymphocytes	41 per cent.
Hæmoglobin	80 per cent.

On December 18 she developed signs of pneumonia and died five days later. The urine showed a moderate amount of albumen with hyaline casts but no Bence Jones protein.

X-ray photographs showed atrophy of the cervical vertebræ with destruction and collapse of the third, fourth and fifth. Areas of rarefication were thus demonstrated in many of the other bones including especially the ribs and long bones.

At the *autopsy* there was found a widespread confluent lobular pneumonia. The alterations of especial interest concerned the bones.

The ribs were rarefied and softened to such a degree that in most places they could be crushed easily between the fingers. They were especially thinned and greatly depressed on each side of the sternum and in these broad grooves nearly every one showed several fractures, often marked out by the growths of callus tissue.

The eleventh dorsal vertebra had collapsed to a certain degree, so that there was a slight angular deformity at that point. Three of the cervical vertebral centra had collapsed in the same way, bending the neck forward into a position in which it had become somewhat rigid.

One humerus was cut open and its cortex proved to be not more than 1 mm. in thickness, while the large narrow cavity was devoid of any cancellous lamellæ and filled with a mottled grayish red opaque marrow.

If we may judge from the X-ray photographs the same rarefication had occurred in the other humerus and in the bones of both forearms, as well as the femora tibiæ and fibulæ. No fractures had occurred in those bones and there was no sign of a tumor growth at any place. Many diagnoses were ventured to explain the rarefication of the bones and the many fractures but even at the autopsy the mere cellular character of the marrow exposed in the humerus did not suggest a tumor formation. The cut surfaces of the vertebræ were perfectly homogeneous and except for a slight opacity, not different from normal bones.

MICROSCOPIC FINDINGS

Smears from the marrow stained by Giemsa and Hastings' methods showed a remarkable uniformity of cells. Nearly all were rounded or oval cells of moderate size with rather deeply stained nucleus and practically no granules in the blue stained cytoplasms. There was found a pale halo around the nucleus. Occasional suggestions of basophilic granulations in small numbers were found.

Sections reveal a marrow composed in places of the normal cells in their usual arrangement, but nearly everywhere there were found compact masses of cells of one type pushing aside the normal marrow and extending everywhere into contact with the bone. Many fragments of cancellous lamellæ were found isolated in such masses. This tissue which was composed of cells corresponding with those found to be predominant in smears did not as a rule inpinge directly upon the bone but seemed always separated from it by a thin line of cells flattened almost like endothelial cells, which may have been osteoblasts. Nevertheless the effect in causing the destruction and wasting of the bone was extraordinary.

The cells which make up this tumor tissue were not like the myeloblasts described as forming the tissue in a case with numerous myelomatous tumors published in the *Journal of Experimental Medicine* some years ago (Vol. 6) but resembled more closely plasma cells. Whether they correspond exactly with plasma cells it is difficult to say, but after observing recently two cases in the laboratories of Dr. Norris and Dr. Warren, in which the whole character of the growth was very similar to this except in that definite tumors were found, it seems that two distinct types of myeloma must be recognized. Of these one is composed of myeloblasts, the other of cells practically identical with plasma cells. The explanation of the absence of albumoses in the urine is not clear but there have been recorded several other cases in which the urine was free from these bodies.

AORTIC ANEURYSM PERFORATING SUPERIOR VENA CAVA

W. G. MACCALLUM, M.D.

Clinical History.—H. G., woman, age 33, was admitted to hospital fifteen times between January, 1913, and her last admission, in the summer of 1914.

She had led a dissipated life with much abuse of alcohol and tobacco and very possibly had had syphilis. She entered the hospital complaining of shortness of breath and swelling of heart, face and neck. Ten days before admission in January, 1913, patient felt that her face was swollen; the same night she woke up with a choking sensation accompanied by pain. The neck was much swollen and the face bloated.

The physical examination showed œdema of the upper part of the chest and great dilatation of the capillaries in that region. A tracheal tug was felt. There were diastolic and systolic murmurs over the heart and a definite Corrigan pulse. The veins in the epigastric and hypochondriac regions were dilated and tortuous. Eye grounds normal.

During the next month the cyanosis and subcutaneous œdema in the upper part of the chest increased. There was a good deal of cough with rusty sputum.

In February of 1914 these conditions had become accentuated, marked dyspnœa and continuous murmur heard over the back as low as the fifth dorsal vertebra. There was a large accumulation of fluid in the right chest.

In May the superficial veins over the upper part of the chest were very prominent. Wassermann reaction positive.

June 16, 1914, admitted for the last time with still more exaggerated symptoms of the same character.

Death occurred on August 26.

At the *autopsy* the left lung was found to be a good deal compressed and to lie against the vertebral column. The heart was removed, together with the large vessels and the lungs and upon dissection it shows the following:

The heart itself is large, its walls somewhat thickened; the pulmonary artery is pushed forward by a saccular enlargement of the aorta beneath. This sac measures 5 to 6 cm. in diameter in each direction, so that the pulmonary artery is not really much compressed. The superior vena cava is delicate until it reaches the region of the auricle, where it becomes thickened and somewhat rigid and is adherent to the aortic dilatation. The thoracic portion of the aorta shows no dilatation or intimal change up to the end of the ascending arch. The large vessels of the arch pass off normally. They, like the aorta, are delicate and elastic.

On opening the heart and cutting through the aortic ring and the dilated aorta it is found that the left auricle is normal; the left ventricle dilated; mitral valves normal; its trabeculæ and papillary muscles flattened. Just beneath the aortic valves there are linear rough elevations which look like a beginning reaction to aortic insufficiency. The aortic valves themselves are fairly competent by the water test; their edges, however, are thickened and roll into a cord, so that insufficiency must have been possible, with dilatation, and probably existed all the time. Aortic orifice measures 7 cm. in circumference. The tricuspid and pulmonary valves are normal. Right ventricle is rather large; its walls measure 7 mm. in thickness; left ventricular wall measures 19 mm. in thickness. Coronary orifices are normal. Just above the coronory orifice there is a sudden dilatation of the aorta which ends as suddenly with the precipitous edge at the upper end of the ascending part of the arch. It is nowhere roughened or covered by thrombi, but the inner surface is very irregular and there are numerous small sacculations as if new small aneurysms started out from the wall of the old one. One of these, about 12 mm. in diameter, arises from the lower posterior part of the main aneurysmal sac and extends over toward the superior vena cava and presses against it; indeed, it practically pierces a way through the middle of the vein and divides it into two channels, fusing with the further wall of the vein, so that it looks like a bridge across the cavity of the vena cava, when it is exposed by opening that vessel. The blood was forced to pass on each side of this bridge and the two channels must have been still further narrowed by the thickening of the vein wall at this level. They now measure about 10–15 mm. in diameter. Under such circumstances there was no connection between the aortic lumen and that of the vein, since the aneurysmal saccule merely pushes its way through the middle of the vein to the opposite side; nevertheless it undoubtedly caused a good deal of obstruction to the return of blood from the head.

But the aneurysmal sac is found to have burst laterally as it passed across the vein, and a probe from the main aneurysm into the saccule emerges into the vein through a hole which proves to be in the wall of the bridge turned uppermost. This aperture is surrounded by a thrombus on the venous side and it was obviously through this that the blood rushed.

One receives the impression that this actual perforation may have taken place rather recently but the symptoms which it could have produced, such as murmurs and cyanosis, were already present to such a degree on account of obstruction of the vein and the valvular murmurs that it is surprising that the diagnosis could have been reached so accurately as it was.

PROGNOSIS IN PELLAGRA

A Preliminary Note

J. F. SILER, M.D., P. E. GARRISON, M.D., AND W. J. MACNEAL, M.D.

(From the Robert M. Thompson Pellagra Commission of the New York Post-Graduate Medical School and Hospital)

This is a preliminary report based upon the records of 1,162 cases of pellagra, all the cases which have been ascertained to have existed in Spartanburg County, South Carolina, up to October 15, 1914. We shall consider the prognosis first in regard to death rate in first year of the disease, or the year of initial attack, second in respect to liability of the survivors to have a recurrent attack in subsequent years or to escape further attacks, and third, the death rate in recurrent attacks. In each instance we shall inquire into the influence of race, sex and age upon the prognosis.

The gross death rate in the year of initial attack is shown in Table I.

TABLE I

Death Rate in Initial Attack of Pellagra

Year	Recorded Initial Attacks	Recorded Deaths in Year of Initial Attack	Indicated Death Rate, Per Cent.
Before 1908	56	13	23.2
1908	20	2	10.0
1909	57	15	26.3
1910	141	29	20.6
1911	229	32	14.0
1912	208	27	13.0
1913	242	37	15.3
1914	209	30	14.4
Total..........	1,162	185	15.9

The indicated death rate for the last four years is undoubtedly more accurate than for the earlier years, and we may say that in Spartanburg County about 14 per cent. of pellagrins die in the year of the onset of the disease.

Of the total 1,162 patients, no less than 977, or about 84 per cent., survived the year of the initial attack of pellagra, so far as these records go. Of these there were 179 cases originating in 1914, for whom the record of the first year is incomplete, and there were 101 patients who passed from observation. There remain, therefore, 697 pellagrins concerning whose subsequent histories we have some recorded data. In the aggregate, the records of these 697 patients show 1,037 instances of recurrence in subsequent years and 591 instances of freedom from recurrence, indicating roughly that those who survive one attack of pellagra have about one chance in three of escaping an attack of the disease in the following year. All cases are here considered together. Some have been followed for ten years, others for only one year; some have recurred every year, others have never shown any recurrence; others have passed one or two years in good health and have then suffered recurrent atacks, while others, again, have had one or more recurrences with subsequent freedom from the disease.

Recovery from pellagra is very difficult to recognize with certainty. Of the 711 recorded cases which originated previous to 1913, there are 91 instances of pellagra in the initial year with subsequent freedom from recognizable evidence of the disease for at least two years. These cases may, with some reservation, be designated as instances of recovery after the initial attack. The indicated rate for recovery after one attack is, therefore, 12.8 per cent. This indication must be taken with some reserve, however, because there were recorded 13 patients who again suffered an attack of pellagra after an interval of two years without recognizable evidence of the disease.

The gross death rate in the year of recurrent attack is shown in Table II.

TABLE II

Death Rate in Recurrent Attack of Pellagra

Year	Recorded Recurrent Attacks	Recorded Deaths in Year of Recurrence	Indicated Death Rate, Per Cent.
Before 1908	80	7	8.8
1908	22	2	9.1
1909	34	5	14.7
1910	58	7	12.1
1911	140	29	20.7
1912	226	32	14.2
1913	233	29	12.4
1914	244	19	7.8
Total..........	1,037	130	12.5

The death rate in recurrent attack is apparently somewhat less, but, on the whole, not significantly different from the death rate in the initial attack.

The correlation between race and prognosis in pellagra is indicated in Table III.

TABLE III

Relation of Race to Prognosis in Pellagra

	White Race	Negro Race
Recorded initial attacks	1,010	152
Recorded deaths in initial attack	121	64
Indicated death rate, per cent.	12.0	42.1
Recorded recurrent attacks	970	69
Recorded deaths in recurrent attack	107	23
Indicated death rate, per cent.	11.0	33.3
Recorded cases incident before 1913	630	81
Recoveries (after one attack)	84	7
Indicated recovery rate, per cent.	13.3	8.6
Recorded cases incident before 1912	450	53
Recurrence for three consecutive years	210	19
Frequency of chronic pellagra, per cent.	46.7	35.8

Those cases tabulated as recoveries were free from evidence of the disease for two years subsequent to the year of onset.

It would appear that the negro race in Spartanburg County has suffered much more severely from the acute type of pellagra, showing a much higher death rate, although as we have shown in previous papers, the negro race, as compared with the white race, is very much less frequently attacked by the disease in this county.

The correlation between sex and prognosis in pellagra is indicated in Table IV.

It would appear that the attack of pellagra is somewhat more decisive in males than in females, as the former show a higher death rate in both initial and recurrent attacks and also a higher recovery rate after the initial attack. Chronic recurrent pellagra, on the other hand, is relatively more common in females.

TABLE IV

Relation of Sex to Prognosis in Pellagra

	Females	Males
Recorded initial attacks[1]	816	345
Recorded deaths in initial attack	123	62
Indicated death rate, per cent.	15.1	18.0
Recorded recurrent attacks	757	282
Recorded deaths in recurrent attack	84	46
Indicated death rate, per cent.	11.1	16.3
Recorded cases incident before 1913	501	209
Recoveries after one attack	59	32
Indicated recovery rate, per cent.	11.8	15.3
Recorded cases incident before 1912	365	137
Recurrence for three consecutive years	175	54
Indicated frequency of chronic pellagra, per cent.	47.9	39.4

The correlation between age and prognosis in pellagra is indicated in Table V.

TABLE V

Relation of Age to Prognosis in Pellagra

Age	0–12	12–20	20–40	40–60	60 and Over
Recorded initial attacks[1]	207	86	533	250	67
Recorded deaths in initial attack	6	13	69	60	27
Indicated death rate, per cent.	2.9	15.1	12.9	24.0	40.3
Recorded recurrent attacks[2]	105	51	557	229	91
Recorded deaths in recurrent attack	4	6	60	43	15
Indicated death rate, per cent.	3.8	11.8	10.8	18.8	16.5
Recorded cases incident before 1913	97	61	327	171	42
Recoveries after one attack	31	12	34	13	0
Indicated recovery rate, per cent.	32.0	19.7	10.4	7.6	0.0
Recorded cases incident before 1912	49	44	239	130	31
Recurrence for 3 consecutive years	23	20	124	49	12
Relative frequency of chronic pellagra, per cent.	46.9	45.5	51.9	37.7	38.7

The comparatively good prognosis in children is clearly evident in the table.

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[1] One child has been omitted here because sex was not recorded.

[1] Nineteen cases omitted because age was not recorded.

[2] Four instances of recurrences omitted because age was not recorded.

Perhaps a few words should be said about insanity in pellagra. This topic has recently been discussed by Singer,[1] working in collaboration with us. He found mental disturbance in about 40 per cent. of cases of pellagra, chiefly symptomatic depressions and delirious states due to the intoxication of the disease, most commonly observed in men aged 21 to 40 and women aged 41 to 60. Such mental disturbance indicates a severe attack of the disease, but it clears up completely if the patient survives. Pellagra is much more common in individuals of faulty nervous organization, but chronic insanity or chronic nervous disease, due strictly to the pellagrous intoxication, if it occurs at all, is very uncommon.

AMŒBAS IN THE MOUTHS OF SCHOOL CHILDREN

ANNA WESSELS WILLIAMS, ANNA I. VON SHOLLY, AND CAROLINE ROSENBERG

(*From the Bureau of Laboratories, New York City Health Department*)

Since the announcement last summer by Smith and Barrett that an amœba genus, called *Endamœba* first by Joseph Leidy in 1879, is probably the cause of pyorrhœa alveolaris, and that an amœbicide, emetin, will cure the disease, interest has been stirred anew in the etiology and treatment of septic mouth conditions.

Among others, the Bureau of Laboratories of the New York City Health Department has begun a study of focal mouth infections helped by the interest and financial aid of Dr. Merritt, and this short paper is a first report of one phase of our study.

Soon after our observations were begun, we found that all cases presented to us by the several dentists who are collaborating with us, already had pus pockets formed in the teeth alveoli—that is, they were well-established cases—and when we began to inquire for beginning cases, we learned that the diagnosis of pyorrhœa alveolaris is made by most dentists—as is the diagnosis "trachoma" by most ophthalmologists—after the disease has made decided progress, so we decided to hunt for beginnings in school children.

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[1] Archives of Internal Medicine, 1915, XV, 121.

The school in which we had done most of our intensive trachoma work was chosen for starting our examinations. So far, about 475 children between the ages of 9 and 16 years have had a preliminary examination and, from 150 representative ones, stained smears have been made and examined for amœbas.

The 475 children have been divided roughly into those having healthy-looking gums and those having receding or spongy and bleeding gums. The healthy-looking gums were further divided into those with one or more decayed teeth, and those with apparently sound teeth. Needless to say, we found very few of the latter, that is, of those with apparently healthy gums and sound teeth—about 1 in 8. About one half showed unhealthy gums. The exact figures will be given later.

Results of Smear Examination of 150 Cases.—From most cases two smears were taken. The smears were made as follows: In the bad cases, the teeth and gums were cleaned with a cotton swab dipped in 50 per cent. alcohol; then with the flat end of a hardwood sterile toothpick, material was scraped from the margin of the gums and stroked lightly over a clean glass slide making 3 or 4 streaks. The slide was then fixed while still moist by dropping methyl alcohol on it, and it was ready to send to the laboratory. This was called the superficial smear. A second toothpick was used to get material from beneath the margin of the gums, from which another smear was made and fixed. This was called the deep smear. In the cases with clean teeth and tight healthy-looking gums, the first smear was made before cleaning with alcohol. All of these smears were stained with Giemsa's stain by A. G. Mann of our laboratory, and were examined by us for amœbas. In regard to the identification of amœbas by stained films, I would say that they are more certainly and easily identified by this method than by warm living preparations, though, of course, both methods should be used in studying all of the characteristics of these microorganisms.

Stained by Giemsa, the delicately but definitely reticulated cytoplasm of the amœba stains a clear blue, while the small nucleus shows rounded or irregular masses of red granules and threads, with often a central vacuole-like body. This nucleus without doubt corresponds to that most frequently described for *Entomœba histolytica,* but since we know that amœbas are notoriously variable in their entire morphology, according to their stage of development and to the conditions of growth, no definite opinion should be expressed as to the species of the forms found in these mouths until much further study has been made. The amœbas usually contain masses of undigested materials. They seem to take up very readily leucocytes, red blood cells, and many kinds of bacteria. I have placed a smear under the microscope from one of these cases.

Number and Percentage of Amœbas Found in Each Group of Children

I.	Healthy gums	+ 6 — 14	= 30 per cent. positive
II.	Healthy gums .... Decayed teeth	+ 11 — 11	= 50 per cent. positive
III.	Tartar and Receding gums	+ 37 — 10	= 84 per cent. positive
IV.	Spongy and Bleeding gums	+ 61 — 4	= 94 per cent. positive

+, positive; —, negative.

Of course, we can say nothing definite yet as to the significance of the amœbas in these mouths. Finding them so often in apparently healthy mouths and in such young children does not agree with the statements of Bass and Johns and of Barrett, that they are not found in healthy mouths. They base their statements on the fact that they were not able to demonstrate amœbas in healthy adult mouths. The demonstration by Le Wald before this society in 1907 of amœbas in the mouths of healthy individuals, cannot be discussed in this connection since the condition of the mouths and the ages of those examined were not stated.

Moreover, we have so far made only one examination. Further investigation may show a larger percentage of amœbas constantly in the healthy mouths and a smaller percentage constantly in the unhealthy ones.

But these results as they stand are very striking, especially when we must add that most of the cases of spongy and bleeding gums show more amœbas in the films than the other cases. If we could only rule out other microorganisms the evidence for the pathogenic action of the amœbas would be strong.

We have, however, a marked opportunity to test the prophylactic effect of ipecac or its emetin alkaloid.

We propose to divide the cases showing amœbas into groups, one to be watched without treatment and others to be treated in different ways. We shall have a tooth wash made up containing a dilution of one of these drugs, and have the cleaning of the teeth done morning and afternoon in the school clinic under the supervision of the school nurse. We hope by combining these practical investigations with the laboratory studies that we may be able to help demonstrate the relationship of these amœbas to septic mouth infections.

Discussion:

Dr. Merritt: I had not the slightest idea of being asked to speak on this subject. I have for a long time been interested in the question of the etiology of pyorrhœa. We had very positive statements made in Philadelphia last July regarding the relation of amœbæ to the disease which has puzzled many practitioners. We thought we would get the research laboratory to find out how much truth there was in the matter. Bass and Johns have made such positive statements that it is important to know whether they are true or not. The indiscriminate use of emetin, too, ought to be checked.

I do not think that the etiology of pyorrhœa depends on endamœbæ. The numbers of amœbæ found, I do not think, bear any relation to the advance of pyorrhœa. The most advanced cases I have seen had the least and the most simple one, most. The more healthy mouths as a rule seem to have amœbæ. The fact that so many are found in the mouths of children is perhaps the best evidence that they have little to do with the etiology of pyorrhœa. Pyorrhœa is certainly not a children's disease. I have seen reports of cases in children, but personally have never seen the disease in a person under 18 years. Amœbæ are found not only in mouths not kept very clean, but also in mouths carefully kept.

Dr. Le Wald: Some years ago I found amœbæ in the mouth of a dentist extremely careful of his teeth. This discovery was made in the examination of the mouths of a large number of cases in the Philippines. The natives showed amœbæ in 75 per cent.; Americans, 71 per cent. In negative cases a repeated examination often resulted in positive findings. Thus I concluded that amœbæ were present in all mouths. Some of these people had carious teeth but not all. I concluded that amœbæ were constant inhabitants of the mouth. I have seen no reason to change that opinion.

Dr. Zinsser: In examining pyorrhœa cases we have found a great many spirochætes where the pus was taken from the gums. I think that is because of the presence of necrotic tissue, as in Vincent's angina.

Dr. Williams: I should like to emphasize the question of the beginnings of the disease. Dr. Merritt says the earliest case he knows was 18 years. These children were from 9 to 16 years. Sixty-five of them had spongy, bleeding gums, slightly receding, with a great accumulation of leucocytes. The question is, are some of these beginning cases of pyorrhœa alveolaris?

Dr. Merritt: While there is usually a mild gingivitis in pyorrhœa, the disease affects essentially the alveolar bone. I, therefore, do not regard Dr. Williams' cases as true pyorrhœa. I think with proper care of the teeth pyorrhœa would never develop. One may have considerable loss of bone from the abrasive action of the tooth brush with no disease, or pyorrhœa with considerable loss of bone in addition to the gingival condition which accompanies it.

Dr. Krumwiede: I believe Dr. Williams found in these children as well as in pyorrhœa cases in adults enormous numbers of spirochætes with the amœba.

Dr. Williams: Yes, in practically all mouths of these children except the most clean there were large numbers of spirochætes as well as many other varieties of microörganisms.

A BRILLIANT GREEN AGAR FOR THE ISOLATION OF TYPHOID BACILLI FROM FÆCES

CHARLES KRUMWIEDE, JR., M.D.

(From the Division of Laboratories, Department of Health, New York, N. Y.)

During the last year we have accumulated a great deal of data concerning the action of brilliant green on the growth of members of the typhoid-paratyphoid-colon group, when the dye is used in fluid and solid media. We hope to report the data shortly. At this time, I shall only describe a new plating medium for the isolation of the typhoid bacillus from stools.

The basis for the medium is an agar consisting of water, 1,000 c.c., Liebig's extract of beef, 3 grams, peptone, 10 grams, salt, 5 grams, and agar 15 grams. As this agar is used also as the basis for Kendall's modification of the Endo medium, we have found it most convenient to have the reaction made slightly alkaline to litmus, and to keep the medium in bottles of 100 c.c. amounts. To 100 c.c. of the melted agar is added 1 c.c. of the Andrade indicator (consisting of 100 c.c. of a 0.5 per cent. watery solution of acid fuchsin decolorized by the addition of 16 c.c. of normal soda solution). The indicator keeps well and is not affected by light. Normal acid is then added till the agar is distinctly red. The correct point is reached when the hot agar is red but the color disappears on cooling. Having determined the correct amount for one bottle, this is noted and a similar correction made for the bottles of this batch of agar as used. This method has the advantage over setting the reaction of the agar in bulk in that variations are possible, should they seem necessary.

One per cent. of lactose and 0.1 per cent. of glucose are then added, using sterile 25 per cent. solutions of the sugars. After mixing, the brilliant green is added. We are now using two concentrations of the dye. To one bottle is added 0.2 c.c. of a 0.1 per cent. solution, giving a final dilution of 1 : 500,000, and to another is added 0.3 c.c., giving a dilution of 1 : 330,000. Each bottle is sufficient for six plates, as the layer of agar must not be too thin. The plates are left uncovered until the agar is hard, and then covered with porous tops. The medium is inoculated by surface streaking.

On this medium, the typhoid colony is very characteristic. With the light passing obliquely through the plate, they have a peculiar snow-flake appearance. Using a hand-lens with artificial light, they have the texture of a coarse wool fabric.

The brilliant green inhibits the growth of many of the bacteria in the feces, and in this way gives a relative enrichment of the typhoid bacilli, if present. The degree of inhibition of the fecal types depends on the flora and varies widely.

Two dilutions of the dye are used to obtain the maximum inhibition of the fecal flora and still insure good growth of the typhoid bacilli, which vary somewhat in their reaction to the dye. Endo plates should be planted as well. Although we have never failed to isolate typhoid bacilli from the green dye agar when they were present on the Endo medium, a much greater experience is necessary before we can be sure that some strains of typhoid may not be exceptionally sensitive to the dye and therefore fail to grow. Any change in the constituents of the medium may upset the balance, and require a readjustment of the amount of necessary dye.

Testing the medium by adding traces of carrier stools to normal stools, we obtained the following results. Of 130 such mixtures, 71 gave negative results on Endo. Of these, 48, or 67 per cent., were positive on the green dye agar containing 1 : 500,000 of the dye. In another series of 28 stools, both strengths of dye were used. Of these stools, 18 were negative and 10 positive on Endo; 9 were negative and 19 positive on dye agar containing 0.2 c.c. and 3 were negative and 25 positive on dye agar containing 0.3 c.c. One gave negative results with dye agar containing 0.3 c.c. due to almost complete inhibition of growth, but was positive on dye agar containing 0.2 c.c. Nine stools obtained for diagnosis during a localized epidemic gave similar results. All were negative on Endo, 4 were positive on the dye agar containing 0.2 c.c. and 5 on the dye agar containing 0.3 c.c.

Discussion:

Dr. Zinsser: In the preparation of typhoid media one difficulty we have found is the uniformity of production of media. In the green media is there any difficulty in getting uniform preparations? Is the dye constant?

Dr. Krumwiede: We have used the preparation of one manufacturer. Brilliant green, extra crystals, No. 366, Bayer. It is more uniform than the malachite green.

Dr. Zinsser: How is the media titrated?

Dr. Krumwiede: We have made the medium alkaline to litmus. The Andrade indicator gives a red color in hot media and is colorless when cold, when the correct point is reached; as a rule, 5 normal Hel. is required to bring it to that point when originally alkaline to litmus.

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Dr. Hans Zinsser, *President*

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS[1]

I. BACTERIOLOGICAL STUDIES

HARRY PLOTZ, M.D.

Abstract[2]

In a preliminary communication published in the *Journal of the American Medical Association,* May 16, 1914, I described an organism which I considered to be the etiological agent in typhus exanthematicus. The organism, a bacillus, was recovered from the blood of five cases of European epidemic typhus fever and of six cases of the mild endemic form of the disease known in the

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[1] From the Pathological Laboratory, Mount Sinai Hospital, New York. For the financial support of these investigations, we are indebted to two contributors who desire to remain anonymous.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases.*

United States as Brill's disease.[3] Since that time I have had the opportunity of studying a much larger series of cases with the same cultural methods, and the results, together with the serological and experimental studies, have confirmed the opinion then expressed. At the present time, we shall give only those data which bear upon the question of etiology, leaving other phases of the work for future reports.

The findings of previous investigators will not be discussed because none corresponds to the organism which I have found.

The differentiation of the mild endemic form of typhus fever seen in New York from other fevers, especially short term typhoids, is due to the clinical insight of Dr. Nathan E. Brill. Its similarity to typhus fever was later noted by Louria and G. A. Friedman. Following this came the striking cross immunity experiments of Anderson and Goldberger, which demonstrated that the disease described by Brill was most likely a mild form of typhus fever.

My studies were begun with the endemic form of the disease, using the viewpoints that had been established regarding typhus fever.

It had been demonstrated by early experiments that the virus exists in the circulating blood during the febrile period of the disease. It was also evident from previous studies that the virus was not filterable. Ricketts and Wilder searched the blood microscopically and saw a bacillus which they described. There is no evidence that the bacillus seen by them is the same as that which forms the basis of this report. Fried and Sophian could find no organisms in smears of blood made from five cases of endemic typhus fever.

It was surmised that because typhus fever had been proved to be an insect-borne disease, a protozoan origin would needs be found, but Ricketts and Wilder, in refutation, pointed out that the organism could be bacterial.

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[3] As these studies give the definite proof that the cases of European typhus fever and the cases of "Brill's disease" are due to the same organism, we shall designate the European cases, *epidemic* typhus fever, and the New York cases, *endemic* typhus fever.

Aerobic cultures of the blood had been made by a number of observers, but as these had all been negative, I decided to use anaerobic methods.

Up to the present time, eleven cases of European epidemic typhus fever and forty cases of the local endemic form of the disease have been studied. I am indebted to Dr. Joseph J. O'Connell, Health Officer of the port of New York, for the privilege of studying the epidemic typhus cases and to the attending physicians of Mount Sinai Hospital for studying thirty-six of the forty endemic cases.

This work, as well as the investigations which form the basis of the subsequent papers, was carried out under the direction and with the helpful assistance of Dr. E. Libman. It was the energy and stimulus which he supplied to the work which helped us to carry it to a successful conclusion.

We wish also to thank Dr. F. S. Mandlebaum, pathologist of the hospital, for many courtesies and for placing all the facilities of the laboratory at our disposal.

METHODS OF CULTURE

Aerobic Methods.—Fifteen cubic centimeters of blood are withdrawn and inoculated into flasks of plain bouillon and glucose bouillon and tubes of agar and glucose agar, plates being made from the latter.

Because of the report made by Rabinowitch that he had obtained positive blood cultures in cases of typhus fever by the use of an aerobic method, his medium was tested. The results were negative in all the cases tested although some of these cases gave positive results with the anaerobic method.

Anaerobic Methods.—The first successful results were obtained in two cases of endemic, and one case of epidemic typhus fever by the use of the ascitic fluid-kidney tissue medium, layered with liquid petrolatum, as advocated by Dr. Noguchi for the cultivation of spirochetae. I am indebted to Dr. Noguchi for a demonstration of his methods. The method introduced by Liborius-Veillon proved to be more satisfactory,—modified in that serum-glucose-agar, originally suggested by Libman as an optimum medium, was used instead of glucose agar.[1]

Serum Employed.—The sterility of the fluid must be ascertained before use, by thorough aerobic and anaerobic methods, such as will be described in the full publication. Studies must also be made to exclude hemoglobinophilic organisms.

DEVELOPMENT OF THE ORGANISM OBTAINED: SUBINOCULATIONS

Colonies of the organism appear in the tubes in from three to sixteen days. Negative tubes were observed twenty-one days. On development, the colonies are removed and inoculated on slants of one half per cent. glucose-serum-agar, the latter tubes then being inserted in Buchner tubes.

The colonies usually appear in the lower two or three centimeters of the tube, occasionally also higher, but never within three centimeters from the top of the medium. The colony first appears as a small opaque spot. As it grows larger a brownish area of precipitation[2] develops in the medium about it. By direct sunlight or incandescent electric light (frosted bulb) the colony itself appears round and opaque, varying in size from one to six millimeters in diameter (including the area of precipitation), the size of the colony depending on its stage of development. On cross section it is Y-shaped, brownish in appearance, and soft in consistency. The arms of the Y are fusiform.

MORPHOLOGY

The organism is a small pleomorphic Gram-positive bacillus, which is non-motile, non-encapsulated and not acid fast. Its length varies from 0.9 to 1.93 micra, its breadth being from one fifth to three fifths the length. Most of the organisms are straight, occasional ones are slightly curved. Coccoid forms also occur. The ends are rounded or slightly pointed. In smears from solid media, the organisms lie end to end, side to side or at angles to one another, there being no definite arrangement. Degeneration and involution forms appear early. The organism produces no spores. Polar bodies are occasionally demonstrable either at one end of the bacillus or both.

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[1] The details of the method will be described in the full publication.

[2] This phenomenon, which is due to acid production, was first described by Libman in 1901, when he advised the use of media containing carbohydrates and sera (non-coagulated) for the growth of poorly growing organisms.

CULTURAL CHARACTERISTICS

Besides the usual studies of cultures grown in Buchner tubes or in Novy jars, inoculations were made in a variety of media in long test tubes. A saline emulsion of the organism was inoculated in the following media:

Agar,
½ per cent. glucose agar,
2 per cent. glucose agar,
Sugar-free bouillon,
2 per cent. glucose bouillon.

These media were used alone; layered with liquid petrolatum, with the addition of kidney tissue; with ascitic fluid; with ascitic fluid and kidney tissue; and with ascitic fluid and kidney tissue layered with liquid petrolatum.

There was no growth in the agar or sugar-free bouillon after twenty days observation. Growth was present in one half per cent. glucose-agar with ascitic fluid and kidney tissue, but was better in two per cent. glucose-agar. There is a minute flocculent growth at the bottom of the glucose-bouillon tube after eight days.

In Buchner tubes, after three days, on glucose-serum-agar there is a creamy white growth which does not spread and which in older cultures may become light brown in color. There is moderate precipitation which increases after three days and increases on subculture.

On potato, after four days there is a practically invisible whitish growth demonstrable by scraping the medium. On gelatin, fifteen per cent., there is no growth even in the thermostat. On litmus milk, there is a slight amount of acidity after eighteen days. On Loeffler medium, there is very slight growth.

The organism from both the epidemic and endemic cases was found to produce acid and precipitation in glucose, maltose, galactose and inulin and not in raffinose, mannite, arabinose, saccharose, dextrin and lactose. The thermal death point was found to be 55° C. for ten minutes. The organism was found to be non-filterable.

On plates, colonies could be developed only in glucose-serum-agar, appearing after seven days. Surface colonies were obtained only once. The deep colonies varied in size from pinpoint to one millimeter. They were round, oval or triangular, the last type predominating. Microscopically, the colonies were triangular or acorn-shaped, sharply circumscribed with irregular contour.

Aerobic Studies.—The organism is an obligatory anaerobe. It was at first thought that slight growth had occurred aerobically, after anaerobic cultivation. The strains were inoculated on a variety of aerobic media, with negative results.

GENERAL RESULTS OF THE BLOOD CULTURE STUDIES

Blood cultures were taken in seven cases of epidemic typhus fever during the febrile stage and the bacillus isolated in each.[1] From an eighth case blood was inoculated intraperitoneally into two guinea-pigs which developed the typical febrile reaction and from the blood of which at the height of the reaction the organism was obtained.

Blood cultures were taken in thirty-four endemic cases up to the time of crisis. Eighteen of these, or fifty-three per cent., yielded the bacillus.

Blood cultures were taken after the crisis in six of the cases of epidemic typhus fever and all proved negative. In three of these the organisms had been recovered during the febrile period of the disease. Nine of the endemic cases were cultured after the crisis and the bacillus was found in one of them twelve, and in the other, thirty-six hours after the crisis.

With the identical technic used for isolating the bacillus in typhus fever, anaerobic blood cultures were taken on one hundred and ninety-eight control cases and in no instance was an organism recovered which in any way resembled the bacillus recovered from cases of typhus fever.

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[1] In the full paper these facts will be elucidated by tables giving all the details.

THE IDENTITY OF THE ORGANISMS ISOLATED FROM VARIOUS SOURCES

The organisms isolated from the endemic typhus cases were found to be culturally identical with those isolated from the epidemic typhus cases. Not the slightest variation was found. The organisms isolated from guinea-pigs and monkeys in which the disease had been produced experimentally were also exactly the same.

A careful study of the facts in the tables brought out the interesting observation that the organism occurs more abundantly in the blood on the fourth and fifth days before the crisis than later. It was also found that the bacillus was found eighteen times as abundantly in the blood of the epidemic as in the blood of the endemic cases. Without laying too much stress on this exact figure, it is very evident that the bacteriemia is more intense in the epidemic than in the endemic cases. The two cases of epidemic typhus fever which had the most marked bacteriemia proved fatal.

Because of the late maturation of the colonies, the results of the blood cultures are usually only known after the termination of the illness and therefore can be used only for confirming a previously made diagnosis, but in two instances of supposed influenza, the organism was found after the crisis and then a careful investigation revealed the fact that both of the individuals had passed through an attack of typical typhus fever.

CONCLUSIONS

Attention has been drawn to the fact that the virus of typhus fever circulates in the blood of an infected person or animal, that the virus is not filterable and, therefore, of microscopic size; and that aerobic cultures made with a great variety of media are negative. Hence it was considered plausible that the infective agent is an anaerobic organism. By means of an anaerobic method of blood culture a bacillus was isolated in pure culture from seven cases of European epidemic typhus fever, or one hundred per cent. of the cases studied during the febrile period. With the same method the identical organism was recovered during the febrile period of the local endemic form of the disease, in eighteen of thirty-four cases, or fifty-three per cent., and in two additional cases taken after the crisis. Similar blood cultures made in one hundred and ninety-eight control cases yielded no such organism.

Considered in conjunction with the serological and experimental evidence which follows, these facts prove that this bacillus is the etiological factor in typhus exanthematicus.

Following a very kind suggestion of Professor William H. Welch, it has been deemed advisable to name the organism, *Bacillus typhi-exanthematici.*[1]

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS

II. SEROLOGICAL STUDIES[1]

PETER K. OLITSKY, M.D.

Abstract[2]

I. COMPLEMENT FIXATION DURING THE COURSE OF TYPHUS FEVER

In the beginning three antigens were used, one made up of the epidemic typhus organisms, one of the organisms of endemic cases, and a third of a mixture of both organisms. Later it was

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[1] The name *Bacillus typhi exanthematici* was applied by Klebs in 1881 to a hypothetical organism in typhus fever. The name was never actually used, as the search for the organism proved futile. The hyphenating of "typhi" and "exanthematici" succeeds in giving us a binomial designation properly descriptive.

[1] This work was done under the tenure of the Eugene Meyer, Jr., Fellowship.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases.*

found better to use an antigen made up of both strains of the typhus bacillus. The results were then found to be more uniform, and positive reactions more common.

Eleven cases of epidemic and thirty-four of endemic typhus fever were tested. Of twenty-five cases studied at the height of the disease, only two were positive, one giving a ++ reaction, the other a ++++ reaction. Both were tested one day before the crisis. The latter had a negative, the former a positive blood culture. Ten cases were tested at the crisis. Of these, two gave ++++ reactions, one a +++, one a ++, and the rest were negative. In the post critical stage of the disease, nine epidemic and thirty endemic cases were studied. Of these, 71.8 per cent. gave positive reactions.

The complement fixing antibodies reach their maximum concentration between the second and twelfth day after the crisis. Their persistence was studied in two cases, in one of which the reaction became negative between the sixty-fourth and the one hundred and thirteenth day after the crisis, and in the other case between the third and thirty-ninth day after the crisis. One may trace the development in an individual case of a negative reaction at the height of the disease to a ++++ reaction after the crisis.

One hundred and four control cases were studied with negative results.

Complement Fixation on Typhus Fever Cases with other than Typhus Antigens.—These antigens were made of *Bacterium coli, Staphylococcus aureus, gonococcus, streptococcus* (single antigens made of various types) and some Gram-positive anaerobic bacilli, having a morphology similar to that of the typhus bacillus (*Bacillus acnes* and bacilli isolated from ascitic fluid and from rabbit's kidney). These antigens were prepared in the same manner as the typhus antigen.

2. AGGLUTINATION REACTIONS DURING THE COURSE OF TYPHUS FEVER

Microscopical reactions were found to be better than macroscopical. Comparative experiments demonstrated that an epidemic typhus agglutinogen gave clumping with serum from endemic as well as from epidemic typhus fever, and an endemic typhus agglutinogen likewise gave positive agglutination with serum from patients suffering from either form of the disease.

In applying a mixed agglutinogen, positive agglutinations were obtained in more instances than positive complement fixation tests. For reasons which we will give later, only reactions in dilutions of 1 to 50 or higher were considered positive.

In forty-three cases of typhus fever during some period in the disease, thirty-nine, or 90.8 per cent., were found positive. Of twenty-four cases tested all were negative, except two which were tested a day before the crisis; both had a titer of 1 to 100. Of ten cases tested on the day of crisis, three were positive and seven were negative. In the apyrexial period, thirty-eight cases were studied of which only three were negative and 92.1 per cent. positive. The agglutinins evidently follow a curve similar to that of the complement fixing antibodies, the maximum concentration developing about four days after the crisis. The agglutinins have been demonstrated as late as five months after the crisis.

Agglutination Tests on Control Cases.—There were ninety-six control cases studied at various periods. Seven of these gave an agglutination in dilution of 1 to 20, but not in high dilutions. Of the seven cases one was a brain tumor; two, valvular defects; two, general paresis; one, carcinoma of the tonsil; and one, a case of alcoholism. The complement fixation in all these cases gave a negative result. It is because of the results in these cases that we regarded only such results as positive that gave agglutination in dilutions of 1 to 50 or higher.

There were three control cases in which the occurrence of a previous attack of typhus fever could not be excluded, in which reactions were found in dilutions of 1 to 100 to 1 to 200.

Agglutination with other than Typhus Organisms.—We endeavored to use as an agglutinogen all such bacteria as we were able to obtain in cases other than typhus fever, which showed any resemblance, morphologically, to the typhus bacillus, including the *Bacilus acnes.* In all such instances, when the agglutinogen was mixed with convalescent typhus serum, the results were negative.

3. PRECIPITATION REACTIONS IN TYPHUS FEVER

Of ten cases studied at the height of the disease all were negative. Of three cases tested at the crisis, one showed distinct clouding in 1 to 1,000 dilution. Fourteen, or 73.6 per cent. of nineteen cases tested in the fever-free period, gave precipitations. Three of these reacted in dilutions of 1 to 1,500; four in dilutions of 1 to 1,000, while the rest showed precipitates in dilutions of 1 to 100.

With each test a negative serum was included. Such control cases always gave negative reactions. The precipitin formation is evidently absent at the height of the disease but becomes in evidence at the crisis and proceeds to increase until well along in the post critical stage.

4. BACTERIOTROPINS

While discrepancies are common in making such studies, the average results demonstrate that the opsonic index increases at the crisis and remains high in the convalescent stage of the disease. Similar rises were noted in artificially immunized serum (rabbit). The probability is that this method is one of the most potent on the part of the patient in overcoming the infection. We noted during these examinations that phagocytized bacteria had a marked tendency to become fragmented and take the stain very lightly. Lysis of the typhus bacilli apparently is very active within polymorphonuclear cells of typhus immune subjects.

Other features of this work, the occurrence of allergic reactions, and the nature of the toxin produced, will be reported later.

5. THE IDENTITY OF THE ORGANISM ISOLATED FROM CASES OF ENDEMIC TYPHUS FEVER TO THAT ISOLATED FROM CASES OF EPIDEMIC TYPHUS FEVER

For the purpose of investigating this subject, cross fixation investigations were made both in artificially produced immune serum as well as in natural immune serum and similarly by cross agglutination tests. From studies of rabbit's immune sera the conclusion is definite that from a serological viewpoint the organism obtained from epidemic and the one from endemic typhus fever are two strains of the same bacterium.[1]

6. SEROLOGICAL STUDIES IN ANIMALS

Monkeys.—Of six monkeys that reacted to the typhus virus, complement fixation and agglutination tests were positive in five, or 83.3 per cent. These reactions were still positive twenty days after the crisis.

As the agglutination (Widal) reaction in typhoid fever is no absolute sign of protection from that disease, so in typhus fever, the serological manifestations of complement fixation and agglutination, while often appearing in connection with immunity, are no indications of immunity. The four monkeys mentioned above, two with reactions and two without, were injected with active typhus virus. Three of the monkeys reacted, one did not.[2]

We shall report in the future our results in prophylactic vaccination against typhus fever, a work which is now in progress.

Guinea pigs.—Although these animals were proved to be immune after reacting to typhus virus, the serum contained no agglutinin or complement fixing bodies. It is most probable that this animal develops its high grade of immunity by means of its tissue elements and only to a very slight degree by means of the circulating blood.

There is a possibility that bacteriotropins may exert a great influence in these animals and likewise add to the factors of the cellular immunity which may be present.

Rabbits.—Rabbits are not susceptible to the typhus bacillus in small amounts. When small yet increasing amounts of bacteria are given, they develop very potent immune serum.

[1] See tables in full publication.

[2] The production of complement fixing bodies as well as agglutinins in monkeys can be paralleled in man.

CONCLUSIONS

A careful examination of the above results leads one to the belief that there is most intimate relationship between the typhus bacillus and typhus fever. All the serological reactions occurred in orderly manner, usually becoming demonstrable at the time of the crisis and increasing in concentration after the crisis. There is but one conclusion possible: Typhus fever is a reaction against the organism which has been isolated by Dr. Plotz. The presence of the reactions does not indicate absolute immunity.

As regards the relationship of the occurrence of the reactions to the results of the blood cultures, these facts are noted:

There were sixteen cases in which the blood culture was taken at the height of the disease and in which bacteria were not found. By examining the table which is included in the full publication, it will be seen that most of our strongest serological reactions occurred in just such cases.

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS

III. EXPERIMENTAL STUDIES[1]

GEORGE BAEHR, M.D., HARRY PLOTZ, M.D., AND
PETER K. OLITSKY, M.D.

Abstract[2]

For the purpose of ascertaining whether the organism recovered from the blood of typhus fever patients was also found in animals having experimental typhus fever, a series of twenty-four guinea pigs was inoculated with defibrinated blood obtained from patients or animals with the disease. Blood for cultures was obtained by direct aspiration of the heart sometime after the onset of the fever. From eight, or thirty-three and one third per cent. of the guinea pigs in this series, the same bacillus was isolated from the blood as had been obtained from the blood of individuals with typhus fever. The percentage of positive blood cultures is less than that obtained in human cases because we were forced to use very small quantities of blood for the cultures, the average amount being three cubic centimeters.

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[1] This work was done under the tenure of the Eugene Meyer, Jr., and Moses Heineman Fellowships in Pathology.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases.*

In the positive blood cultures the colonies averaged about one per cubic centimeter of blood. This is more than was found in the patients with endemic typhus fever, but less than in individuals with epidemic typhus.

In only one of nine guinea pigs having mild febrile reactions was the blood culture positive. In fifteen animals with severe reactions the blood culture was positive in seven. This relationship between the blood culture results and the severity of the disease was also observed in human typhus cases.

A majority of the blood cultures were positive when taken between twenty-four and seventy-two hours after the onset of the fever. This observation assumes especial interest when the temperature curves of thirty-five other animals which were permitted to live for the entire course of their disease are analyzed. In the majority of instances (sixty-six per cent.) the fever reached its highest point on the second or third day of the disease, in other words between twenty-four to seventy-two hours after the onset. This is the period when the blood culture is most frequently positive in the infected animals.

Two strains of bacilli obtained from two of the epidemic typhus cases (epidemic cases 1 and 5) were inoculated intraperitoneally into guinea pigs. During the first twenty-four hours after the inoculation both animals developed a slight transient elevation in temperature, after which it remained normal for six and eighteen days respectively. The temperature then rose suddenly to 104° F. (40° C.) at which level it continued. One animal lived during the entire course of the disease, the fever lasting six days and ending abruptly. On the third day of the disease in the other guinea pig a blood culture was made. Seven days later a characteristic colony appeared in one of the culture tubes. This organism upon subculture proved to be both morphologically and culturally identical with *Bacillus typhi-exanthematici.*

Other guinea pigs and monkeys were subsequently inoculated with the two strains of bacilli used in the above experiments or with strains from three other epidemic cases after they had been on artificial media (glucose-serum-agar) for more than three or four weeks, and all strains were then found to have completely lost their virulence. In each instance the inoculation was followed by only a slight transitory rise in temperature during the first thirty-six hours, a reaction also observed in the experiments with virulent organisms and apparently due to a toxin action. After cultivation on artificial media for more than a month, all strains of epidemic organisms also lost this toxin action.

This rapid loss of virulence explains why the reproduction of typhus fever in animals by inoculation of the bacilli was not done more extensively. The positive blood cultures in cases of epidemic typhus fever were obtained at the very beginning of the work. Subsequently when the importance of inoculating the organisms into animals within the shortest possible time after isolation was realized, no more cases of epidemic typhus fever were available.

We hope to have the opportunity very shortly to amplify this phase of the work. It is important, however, that we have demonstrated that with bacilli recently isolated from epidemic cases of typhus fever the disease can be reproduced in animals and that at the height of disease so produced the identical organism can again be recovered from the blood.

Experiments carried out with six strains of bacilli from the endemic cases (endemic cases 1, 2, 18, 23, 27 and 40) and with those isolated from three animals (guinea pigs 21, 24 and 103) with typhus fever, revealed the fact that they lost their virulence outside the body still more rapidly than did the epidemic typhus strains. Before sufficient growth could be obtained for inoculation, the organisms were on artificial media for two to three weeks, or even longer. When finally ready for inoculation such organisms were not only avirulent, but they did not even possess the power of producing toxic rise in temperature after inoculation, a property only lost by the epidemic typhus organisms after cultivation for over a month.

Both morphologically and culturally the organisms isolated from the epidemic and the endemic type of the disease proved to be identical, and both were found to possess the same specific antigenic properties. This variation in virulence and in toxin production is the only essential difference which we have been able to demonstrate between the organisms derived from the two sources. Its importance lies in that it supplies us with a possible reason for the difference in the degree of bacteriemia and in the severity of the two diseases.

RELATION BETWEEN THE NUMBER OF BACILLI IN TYPHUS BLOOD TO ITS INFECTIVITY

The observations presented thus far in this paper have demonstrated the association of the bacillus recovered from cases of typhus fever with the disease as reproduced in the experimental animals. Further evidence from the experimental standpoint that this organism is the etiological agent in typhus fever was readily obtained by a study of the relation between the number of the bacilli in typhus blood and its infectivity.

A calculation of the average amount of organisms apparently contained in the quantity of blood inoculated into each animal was estimated from the total number of colonies which developed in the blood culture tubes.[1]

The uniformity of the observations on this series of fifty-one animals clearly indicates that typhus blood which contains no bacilli, or only very few bacilli, is *not* infective for animals. Typhus blood in which the bacilli are more numerous is infective. These experiments therefore demonstrated that infectivity is absolutely dependent upon the presence of a sufficient number of these bacilli. This in itself is conclusive evidence of their etiological significance.

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[1] The tabulation of these results and the protocols of the experiments upon which these conclusions are based will be found in the complete paper in *The Journal of Infectious Diseases.*

A study of the serum of individuals who have been exposed to infection by contact with human beings or animals suffering from typhus fever, led to the interesting observation that some of these people develop positive complement fixation and agglutination reactions without having had any clinical evidences of the disease.

CONCLUSIONS

It has been shown that a bacillus, identical with that recovered from patients with typhus fever, can also be isolated from the blood of animals in which the disease has been reproduced by inoculation of typhus blood. In such animals, the frequency of the bacilli in the blood is directly proportional to the severity of the illness. In individual animals it is greatest at the height of the disease.

It has also been shown that with the bacilli isolated from epidemic cases of typhus fever, it is possible to reproduce the disease in animals. And furthermore, at the height of the disease in such animals, the identical organism can be recovered from the circulating blood.

Finally, it has been shown that typhus blood is only infective if it contains a sufficient number of these bacilli.

From these observations and the results of the bacteriological and serological studies, we believe ourselves justified in concluding that this bacterium is the causative agent in typhus exanthematicus.

Discussion of papers by Dr. Plotz, Dr. Olitsky, and Drs. Baehr, Plotz and Olitsky:

DR. NOGUCHI: I am very fortunate indeed to have the opportunity of hearing and discussing the important papers just presented by Drs. Plotz, Olitsky and Baehr this evening, as I had the great privilege of being shown the organism by Dr. Plotz when he first isolated it from typhus cases about a year ago. At that stage of the investigation it was of course too early to form any definite opinion as to the etiological relationship between the organism and the disease in question, but now that further evidences that are required for settling such a problem are brought out in a most strictly scientific and thorough manner, it must be evident to anyone that the causative organism of this mysterious disease has been at last discovered. I was interested to hear that not all ascitic media could be used for the first isolation of the typhus organism, as this agrees with my own experience with the cultivation of spirochætes, poliomyelitic microörganism, rabies virus, etc. The question of active and passive immunity, as well as the distribution of the organism within and without the body of the infected host, will undoubtedly be soon solved. I wish to congratulate Dr. Plotz, Dr. Olitsky and Dr. Baehr for this splendid contribution to the study of communicable diseases.

Dr. Park: When Dr. Plotz first announced his discovery many of us were somewhat skeptical, as it seemed an accident. But to-night it is plain that this investigation of the disease was carefully planned and the discovery was after much preliminary work. Dr. Noguchi's previous studies on anaerobic cultures led up to it. I see very few points that have been left unfinished in the work, but there are two points apparently not brought out which are fundamental. There may not have been time to take them up yet. Is the freshly isolated culture virulent for other animals than the guinea pig and monkey? I think Dr. Baehr said that in rabbits large amounts would cause infection, but are other animals similarly susceptible? Secondly, are the monkey and guinea pig, which have been infected by the fresh cultures and recovered from the attack, immune to the blood from a case of typhus fever in man or monkey at the height of the infection? If these two questions can be answered satisfactorily there is no other evidence required. The work appears to have been splendidly done.

Dr. N. E. Brill: These most brilliant papers afford me in particular the greatest satisfaction, because they settle in my mind that the etiology of typhus fever is at last discovered; because they offer for the first time the absolute proof that the disease described by me, which I was the first to assert might be an attenuated form of typhus fever and which I have always believed was related to typhus fever, ever since the important experimental studies on the cross immunity of these two types of fever, is due to the same organism as is epidemic typhus, and hence that they are identical as Anderson and Goldberger asserted; and because the infective agent just revealed may afford a rational means for prophylaxis and perhaps for the cure of typhus fever. We must accept from the work which has been communicated to us to-night that typhus fever exists clinically in two forms, one the classical, epidemic and virulent variety, and the other the mild, attenuated form to which I had the honor of calling the attention of physicians and which exists continually in New York City and in other cities of the United States. Up to this evening, while I had admitted the relationship of these two forms, I could not agree with the deductions of Anderson and Goldberger which they drew from their experimental work, viz., that their work proved the absolute identity of typhus fever and so-called Brill's disease. The reasons, which they urged justified their conclusion, were that when an animal was infected with Brill's disease it was subsequently immune to infection with typhus blood, and on the other hand that when an animal was infected with typhus fever it became immune to infection by the virus of Brill's disease. In the discussion of this feature of their work presented by me at the meeting of the International Medical Congress held in London in 1913, I presented the following arguments against this reasoning: When an individual is infected by vaccinia, he is for a time immune to small-pox, and on the other hand an individual infected by small-pox is subsequently for a time immune to infection by vaccinia. Are small-pox and vaccinia therefore identical diseases? We will all most likely admit that these two diseases are related but not identical. Again, Metchnikoff and Besredka have shown in their very pretty work on immunity in typhoid fever, that when a monkey is once infected by paratyphoid fever, such monkey is immune to typhoid fever; yet paratyphoid and typhoid are related but not identical diseases. Above all I insisted that it was not justifiable to speak with positiveness on the identity of two diseases, especially when the causative organism which most likely produced the diseases under discussion were absolutely unknown.

I should like to say a few words on the subject of the transmission of typhus fever, though it is hardly possible in the five minutes allowed me by the chairman to discuss any of the many interesting subjects suggested by the papers of the evening. Therefore I may only hint at this subject. Nicolle was the first to show that a monkey could be infected experimentally with typhus fever by means of the bites of body lice which had been permitted to feed on a typhus fever subject. Ever since then it has been accepted that typhus fever is transmitted by the agency of the body louse. Anderson and Goldberger not only confirmed this observation but showed that the same results occurred when head lice were used as the agents of transmission. It is very likely that the epidemic form of typhus fever is spread in this way. It would appear from our own observations that this means of transmission is not important in the mild or endemic form of the disease, and that there is most probably some other mode of transmission. These observations are as follows: As was shown by Nicolle, the body louse cannot live at higher temperatures than 12° to 14° C.; it thrives only in the cold when it is most active and presumably most virulent. Anderson and Goldberger showed that when lice were collected and kept in a test-tube in the pocket of the experimenter they died even at such a moderate degree of warmth. It is true that epidemic typhus occurs most frequently in the colder months, as anybody may see who consults the statistics of Murchison, who states that out of 18,230 cases of epidemic typhus fever observed in the London Fever Hospital between the years 1847 to 1870, only 3,730 occurred in the warm months of the year; that July and August furnished by far the least of these. In other words, only about one fifth of typhus fever cases appeared in the summer months. Contrary to the seasonal preference observed in the epidemic variety, the form of the disease which the authors have called the endemic form, shows that by far the largest number of cases occur just in the hottest months of the year, months in which the body louse is not at all active. All my papers show this feature; in fact in the last reported series at the International Congress, of 62 cases reported in 1911 and 1912, 42 occurred in the warm months; 28 of these appeared in the months of July, August and September. The warm months from June to November in our cases furnished twice as many cases as all the rest of the year. Again, if the body louse or even the head louse were the infecting agent in the mild form of the disease, how is it that no family infection appears, that the husband is most rarely infected by the wife, or the wife very rarely by the husband? In over 400 cases of this form of the disease, there has been only one case in which the husband and wife were infected, one by the other. This is a feature which it seems to me requires further study and investigation, for it seems likely that there is some other means of transmission than by the vermin just accused. Finally before I sit down I want to pay my tribute of congratulations to the authors of this evening's epoch-making work in our knowledge of typhus fever.

Dr. Meltzer: I cannot add anything of value to the discussion. I am not a bacteriologist and feel that I am not entitled to offer a statement to the effect that the three communications offered to us prove conclusively that the organism discovered by Dr. Plotz is the real cause of typhus, endemic or epidemic. But since you called upon me, Mr. Chairman, to add something to the discussion, I gladly say this: It gave me great pleasure to listen to the way, the scientific manner and the thoroughness in which these investigations were carried out, and that I greatly appreciate the scholarly and lucid manner in which the results were presented to us. We may congratulate ourselves on the accession to our ranks of this group of young investigators.

Dr. Friedman: I understand that this is not a clinical evening, but a discussion of the origin of typhus. Five years ago I took part in a discussion of Dr. Brill's paper and I must say that I was the first one to state definitely that the symptom complex he described was nothing else but typhus. Hardly anyone then wanted to agree with me. They said they had observed these cases for fourteen years and they were not typhus. Dr. Warren Coleman alone, in a personal communication, said he thought the disease might be a form of typhus. I do not minimize Dr. Brill's work. Men who should know more about typhus than Dr. Brill have made mistakes. In Manchuria, a St. Petersburg professor, Dr. Botkin, found a deviation in type and described it as a different disease. It was clear to me without any bacteriological evidence that Dr. Brill's cases were not different from typhus. In regard to the louse, a Russian physician inoculated himself with the blood of a typhus patient and eighteen days later developed the disease. This inoculation from man to man shows that typhus transmission can occur without the body louse. But I believe the body louse increases the virulence of the disease. This is seen in Russian prisons. Everyone may get typhus from one case, the prisons being filled with lice, and I believe the infection derived through lice is more severe.

Dr. Louria: There is nothing for me to add. From the clinical viewpoint I have to recall cases of typhus fever which I observed in my student days. It is a common disease in Russia. I saw it constantly in my early student and hospital days. When my attention was called to the work of Dr. Brill I was impressed with the fact that these cases looked like what I had seen, but with differences; that is, they run a mild course. Although these two types have been proven bacteriologically and experimentally identical, yet I feel there must still be something missing in the work. I feel, however, that the answers will be presented in time by these able workers.

Dr. Mandlebaum: I should like to draw attention to the great importance of Dr. Brill's work on the isolation of cases of endemic typhus fever—so-called "Brill's disease"—from other fevers. Were it not for his work and the observations that followed, the identity of "Brill's disease" to typhus fever would not likely have been made, and the epidemic cases which were held at quarantine would surely not have been investigated in connection with this work.

When the cause of an acute infectious disease is discovered, one naturally looks for some serum or vaccine for the treatment, or, what is more important, for its prevention. We have heard an allusion made to a prophylactic vaccine. I think I may be permitted to state here that some members of the commission who have already gone to Serbia, and others who intend to go, have been injected at their own request with the vaccine made by us, without any guarantee, of course, as to its efficacy. The efficacy of any vaccine, as we all know, can only be determined when employed in an epidemic.

Dr. Libman: In regard to the question of secondary invaders,—the suggestion has been made that the bacillus described to-night may be an analagous finding to that made in yellow fever by Sanarelli. The latter studies were mostly made after death and in the main mixed cultures were obtained. The work in typhus fever was all done *intra vitam* and the organism was always obtained pure. The serological studies also prove the relationship of the bacillus to typhus fever. If this organism were not accepted it could be only as the basis that there were two etiological agents,—an assumption not warranted by experience.

The organisms when isolated may have lost their virulence because of their long stay in sugar media. If even without sugar they should be proven non-virulent we might make the assumption that the louse is of importance for maintenance and development of virulence.

As to Dr. Brill's remarks on the question of transmission,—either endemic typhus is not conveyed exclusively by lice or we do not yet understand all the features of louse transmission.

The discovery of Dr. Plotz did not depend on chance observation. I have been informed that he had the problem of Brill's disease in his mind when he was a medical student, and that he entered the laboratories of the Mount Sinai Hospital to work on the etiology of the disease. The organism was obtained the first time he tried good methods for it, which is unusual. When he showed me the organism I believed that he had found something of importance and advised him to go on. I consider it a privilege to have been associated with Drs. Plotz, Olitsky and Baehr in the work, although I consider myself more in the character of a watchman than anything else.

Dr. Baehr: Dr. Libman has answered most of the questions. In reply to Dr. Park, we wish to say that we have tested the susceptibility of other laboratory animals, such as rats, mice and rabbits and some observers have tested cats, dogs, sheep and goats. No other animals except monkeys and guinea pigs respond actively to the inoculation of a typhus virus. In reply to Dr. Brill as to the possibility of transmission of the endemic disease by other insects than the body louse,—we prefer not to commit ourselves at present. There may be another agent. Thus far it has only been possible to demonstrate transmission by means of the body louse, and the presence of the latter upon many of the endemic cases of typhus fever, even at the time of their reception in the hospital, is therefore very significant.

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No. 5

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DR. HANS ZINSSER, *President.*

FOREIGN BODIES IN GIANT CELLS[1]

F. C. WOOD, M.D.

(From St. Luke's Hospital, Pathological Department, F. C. Wood, Director)

The patient from whom the material to be shown was removed was a woman (C. B., St. Luke's Path. No. 11022), twenty-six years of age, who about a year previous had had a paraffin injection in the neck for cosmetic purposes. Owing to the considerable hyperplasia which resulted from the injection, it was necessary to remove the mass surgically.

The tissues showed the usual morphological appearance found after such injections, consisting in a very considerable inflammatory reaction of a chronic nature with many lymphocytes and plasma cells lying in a meshwork of granulation tissue. Scattered throughout this granulation tissue were numerous giant cells, some surrounding globules of the paraffin, and others not showing any included structure, while a considerable proportion contained stellate bodies which occupied the center of the cells. These bodies consisted of a central mass, 2 to 3 μ in diameter, from which sprang curved spicules, fifteen to twenty in number. The diameter of the whole body was 20 to 25 μ. These bodies stained fairly well with hematoxylin and somewhat more deeply with Weigert's elastic tissue stain. In the writer's opinion, they probably represent condensed protoplasmic shells about crystals of paraffin, or possibly fatty acids. Mallory and others consider that they are fibrinoid in nature.

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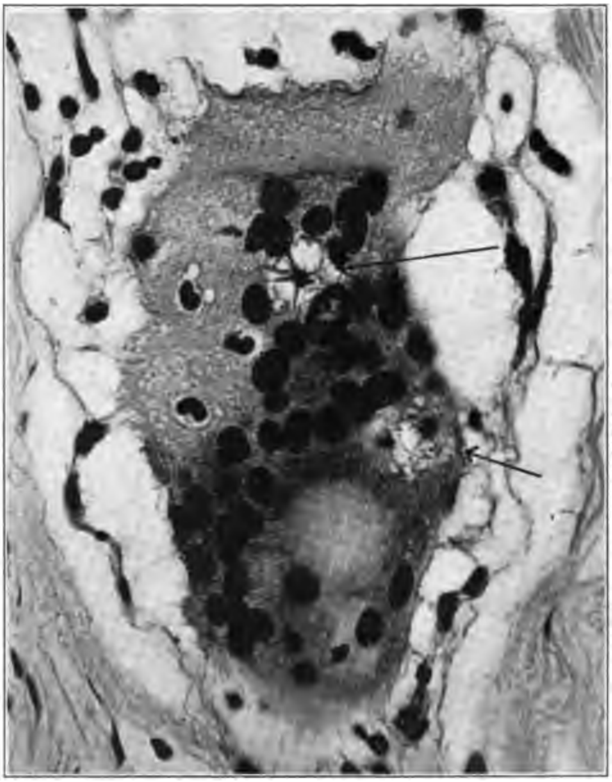
[1] Read at the March meeting of the Society.

The literature concerning these structures, which have, of course, only an academic interest, is not very extensive. The first report was that of Ris, who described similar giant cells in an omental cyst removed from the peritoneal cavity of a man, fifty-one years old. The cyst was about 7 cm. in diameter, with a wall 4 mm. in thickness. The lining of the cyst was composed of granulation tissue with many cholesterin crystal giant cells and also giant cells containing the peculiar stellate structures under discussion. Ribbert, who saw the preparations, thought that they were imperfectly developed portions of moulds (*Schimmelpilze*). These stellate bodies did not stain with the ordinary nuclear stains nor with Weigert's fibrin stain. They measured about 15 to 25 μ in diameter and were composed of curved needles radiating from a central homogeneous area. About one-third of the giant cells contained these bodies.

The next report was that of Wolbach, who stated that in 900 autopsies he had found five cases in which giant cells contained small radiating inclusions. In the first case the structures were present in the lungs; in the second, in the lymph nodes; in the third, in the lungs and lymph nodes; in the fourth, in the spleen, liver, and lymph nodes; and in the fifth, in the lung, spleen, and liver. In one case they were found in the peripheral sinuses of the lymph nodes. The smaller forms were often included in endothelial leucocytes and occasionally within endothelial cells which were attached to the walls of the lymph spaces. Wolbach called attention to the fact that these inclusions were not parasites though they resembled structures occasionally encountered in experimental lesions produced by certain fungi. He held that it was impossible that they should be artefacts. For the material which he studied the best stain was found to be Mallory's phosphotungstic acid hematein; Gram's stain colored the central body and the spines a deep blue, the intervening material taking the counterstain; and with Mallory's eosin-methylene blue the spines and central body were stained a deep purple and the intervening portion a pink color. The bodies were not soluble in caustic potash or in hydrochloric acid, and were not stained by silver nitrate in frozen sections of formalin material. Scharlach R and osmic acid did not stain them nor did iron hematoxylin or Weigert's elastic tissue stain. The iron reaction was negative.

Vogel described similar bodies in giant cells in a case of obliterative capillary bronchitis in a child eleven years old. The giant cells were found in the connective tissue replacing the bronchi and the perivascular connective tissue. They contained one to three star-like bodies which suggested crystals; took a rather faint stain with hematoxylin, and colored best with Weigert's stain for elastic fibers. Fat, fatty acids, and iron could not be demonstrated in the giant cells. Vogel thought that these bodies were probably not altered elastic tissue, but he could give no suggestion as to their nature. He referred to articles by Ssudakewitsch and Rona, but the structures figured by these two authors do not in the least resemble the stellate bodies under discussion.

Iwanzoff found similar structures in a myomatous uterus removed from a woman, fifty years old. In this myoma, which showed a considerable amount of hyaline degeneration, there were areas of lymphoid infiltration containing irregular giant cells. In some cases these giant cells were so abundant that they formed a large part of the nodule. There was nothing peculiar about the giant cells, except that when stained with Weigert's elastic stain they showed peculiar radiating structures previously described by Wolbach. They were not bacterial in nature and did not stain except with Weigert's stain. The writer discusses the question of whether these are phagocyted elastic tissue fibers, such as are frequently seen in tuberculosis of the skin, whether they are parasitic inclusions, or whether they are astrospheres. He referred to a paper by Wakabayashi in which the astrospheres in tuberculous and other giant cells are described, and thought that these bodies closely resembled the structures which he had reported.

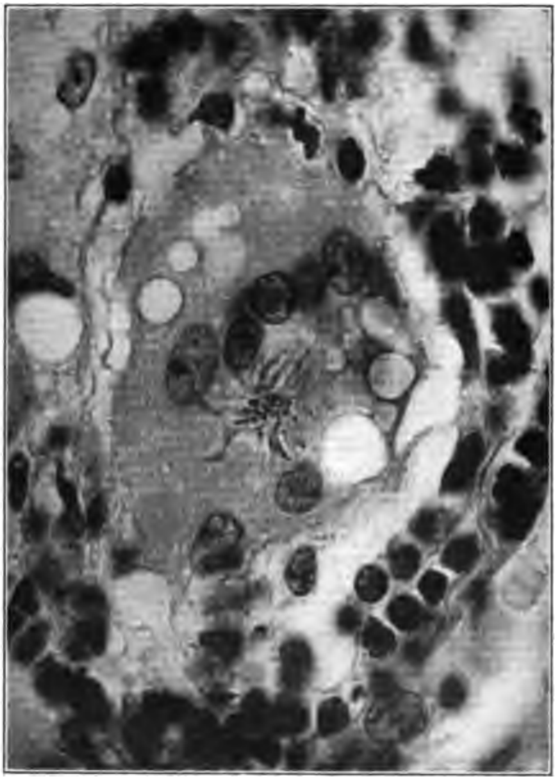


Stellate Bodies in Giant Cells. × 750.

Mallory in his text-book shows a giant cell (Fig. 120, page 207) from a case of leprosy, which contains a spiculated body of the type under discussion. He gives no details except the legend under the cut. In another cut he shows similar spiculated bodies

(Fig. 465, page 613), the text referring to these reading as follows:

"Spiculated bodies have been found in the spleen in a few instances inclosed in endothelial leukocytes and giant-cells. They may be quite numerous and occur in foci or diffusely scattered.



Stellate Bodies in Giant Cells. × 750.

The lesion resembles miliary tuberculosis, but no necrosis is produced. The bodies seem chemically to be of a fibrinoid character. They are evidently not the cause of the lesion, which is probably of infectious origin, but a secondary formation."

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Discussion:

DR. LARKIN: What relation do these bodies bear to the inclusion bodies in cancer?

DR. WOOD: They differ from degenerating cells in carcinoma that I have seen, and I suspect that they may be fatty acid crystals about which condensation of protoplasm has taken place. If they were cellular fragments they would, I think, be more often met with; on the contrary, these stellate bodies are very rare.

CONGENITAL SARCOMA OF VAGINA

A. B. EISENBREY, M.D.

(From St. Luke's Hospital, Pathological Department, F. C. Wood, Director)

In 1911, reports of one hundred and two cases of sarcoma of the vagina were collected by MacFarland of Philadelphia. Of these cases, thirty-four were of the type known as sarcoma botryoides, or grape-like sarcoma. All but one of these cases proved rapidly fatal. They occurred in young children from three months to five years of age. In one case the tumor was noticed at birth. It was shown to be essentially a disease of childhood. On account of the diversity of cell types found it was regarded by many as a congenital tumor.

The case I wish to report was three and one half years of age, a patient in St. Luke's Hospital, on the service of Dr. Abbe. One year previously the family noticed that on crying or making efforts at stools a polypoid mass projected from the vagina. An operation was then performed and the child remained well for about a month, when there was a recurrence. Four operations were performed before her admission to St. Luke's Hospital in March, 1915, at which time the vaginal growth was again removed. While in many cases reported, the tumors were found to be single, the growth in this case was made up of multiple tumors, and involved the entire surface of the vaginal wall. There was apparently some growth also on the margin of the cervix. The child had marked difficulty in micturition and defecation. Palpation through the rectum revealed a sausage-like mass which filled and appeared to be confined to the vagina.

The specimens received for examination were six or eight rounded, pedunculated masses, resembling nasal polyps, pinkish in color, and quite oedematous. In some the pedicle was fibrous. All of the growths were covered by epithelium, which showed a few erosions. Two or three of the small masses consisted of vaginal mucosa containing areas of minute soft nodules beneath the epithelium. Microscopic sections showed a typical polyp-like structure, some of the denser areas consisting of closely packed spindle cells. A few areas of distinctly myxomatous type were found. The predominating cell was of the spindle type. Many young striated muscle fibers were present in some of the polypoid growths, quite apart from the blood vessel walls and the vaginal musculature. They occurred singly and in small bundles. It is on this finding that the "congenital" nature of the growth is based.

Discussion:

Dr. Lambert: I am not at all familiar with the first type of sarcoma of the vagina. On the basis of the microscopic appearance I should hesitate to call it sarcoma. I should rather call it a benign "mixed" tumor or teratoma.

Dr. Eisenbrey: There were a great variety of cells described in connection with this type of tumor. The name was given on account of the grape-like nature of the growths. In some, the clusters seem definitely cystic. Some were myxomatous, but the predominating type of cell was the spindle variety; some showed round cells; some were combinations of the two; three cases were fibromatous in type, others fibromyxomatous; all of these types, however, have been included under sarcoma botryoides. In a large number there were some muscle fibres and in some cartilage. MacFarland's own case did not contain muscle fibers; it was made up entirely of spindle cells, and areas of œdema and degeneration were numerous. They are certainly malignant growths.

Dr. Levin: This tumor seems to be similar to the mixed tumors of the testicle where we find various kinds of cells. They are probably fœtal in origin.

Dr. Eisenbrey: That is the reason I classified this case as a "congenital" tumor.

XANTHOMATA OF TONGUE AND ELBOWS

A. B. EISENBREY, M.D.

(From St. Luke's Hospital, Pathological Department, F. C. Wood, Director)

Xanthoma of Tongue

The specimen was from a man thirty-five years of age, a heavy smoker, a patient in St. Luke's Hospital, on the service of Dr. Abbe, in October, 1914. Two months previously he noticed a hard, painless nodule about 0.6 cm. in diameter on the dorsal surface of the middle of the tongue, slightly to the right side. On section the growth showed distinct demarcation from the surrounding tissue. It was covered by epithelium, and was of firm consistency. Many cells could be scraped from the cut surface. It was whitish in color, and no yellow pigmentation was noticed. Microscopic sections showed groups of characteristic fat-containing cells lying in meshes of a rather dense connective tissue stroma. Under the polarizing microscope the fat proved to be of the double refractile variety.

Xanthoma of Elbows

These specimens were from a young Russian woman, twenty-six years of age, a patient in St. Luke's Hospital, on the service of Dr. Downes. Since childhood she had had yellowish lumps on her elbows. Two months before admission in February, 1915, she noticed the lumps on her elbows were becoming larger, and that some small ones were beginning to appear on both knees. There was no history of previous illness, but the patient was quite neurotic in type, and had physical signs of incipient Graves' disease. Tumors removed from elbows at operation showed well encapsulated, bright yellow, fibrous nodules, which were not attached to the skin. The loose tissue about the nodules and the skin also showed a bright yellow color. Microscopic sections showed typical fat-containing cells lying in meshes of a rather dense fibrous stroma, and also a few fatty cells and fat masses that were loose in the tissue about the nodules. The fat was doubly refractile with the polarizing microscope. Many crystal spaces with giant cells about them were present in some of the dense portions of the nodules.

Discussion:

Dr. Pappenheimer: Are these spaces cholesterin crystals?

Dr. Eisenbrey: Yes. They were considered to be cholesterin and in the fresh state sheaves of fatty acid crystals also were found.

Dr. Lambert: Isn't the tongue an unusual place for xanthoma?

Dr. Eisenbrey: Yes. It is generally found in the abdominal organs, the trachea and pericardium. The small xanthomata about the eyes that occur in old people are probably the result of degenerative processes and not neoplasms. The diagnosis in these cases was made on the nature of the fatty cells and the double refractile nature of the fat to polarized light.

Dr. Lambert: In the second case, I should think there might be a question as to its being xanthoma; occasionally cells containing chlosterin esters are found in other growths. Was the cholesterin content of the blood determined in these cases? Contradictory results regarding this question have been reported. In a recent paper Rosenbloom (Arch. Int. Med., 1913, XII, 395) reported normal cholesterin content of the blood in a case presenting multiple xanthomata.

EOSINOPHILIC INFILTRATION OF THE PANCREAS

HENRY A. RILEY, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, New York City)

The case which is the subject of this report possesses interest in that as far as can be ascertained, it is the only case on record which presents an eosinophilia limited to the pancreas alone and existing in that organ without discoverable cause and independent of a generalized eosinophilia.

Before describing the histological characteristics of the organ involved, it may be well to consider briefly the cause of generalized and local eosinophilia. The main authority consulted is the exhaustive study on general and localized eosinophilia by E. Schwartz in the *Ergebnisse der allgemeine Pathologie,* 1914, Vol. 17, Part I.

General Eosinophilia: Drawing his facts from many sources, Schwartz places the normal limits of the eosinophilic cells in the circulating blood between 2 per cent. and 4 per cent. in adults, 2 per cent. and 5 per cent. in children. Race, sex, age, temperature of the environment, pregnancy and parturition seem to bear no direct influence upon the eosinophile cells. The condition of nourishment appears to exert some influence, starvation reducing the number of cells, which, however, rapidly returns to normal upon the restitution of sufficient nourishment.

Splenectomy or ligation of the splenic vessels is followed by an immediate decrease in the number of cells, then a rise to a supernormal figure and then a slow drop to a subnormal figure which is then maintained.

The effects of many and varied toxic agents have been studied. Those which produce an increase are antimony, CO, CO_2 and pilocarpine. Hæmotoxic substances seem to exert no constant effect upon the number of eosinophiles. Anaphylactic shock is regularly followed by a marked and protracted eosinophilia. This phenomenon is considered to be the explanation of the eosinophilia observed in asthma, hay-fever, many of the dermatoses and certain obscure intestinal phenomena which have been held by many to be due to localized anaphylactic conditions.

Disturbances of the glands of internal secretion do not seem to exert any constant effect upon the eosinophiles except in the thyroid; hypothyroidism being fairly constantly accompanied by an increase, hyperthyroidism by a decrease in the number of eosinophiles. Certain nervous diseases are accompanied by an eosinophilia, among which may be named epilepsy, especially in periods immediately following active seizures, dementia præcox, paranoia, and many psychoses of various origins.

The conditions which produce the most striking examples of eosinophilia are those associated with parasites. The site of the infection does not seem to influence the blood picture, for similar extreme examples are found in all of the groups of parasites whether they exist in the intestinal canal, the parenchymatous organs, the urinary system, the muscles, or the blood. They differ markedly in their eosinophilic properties, however, some of the parasites being unable to stir up any response, while others produce such a profound effect that the eosinophilic cell may occupy an almost exclusive position in the composition of the white elements of the blood. In general, higher values are observed in children than in adults. Those parasites which are

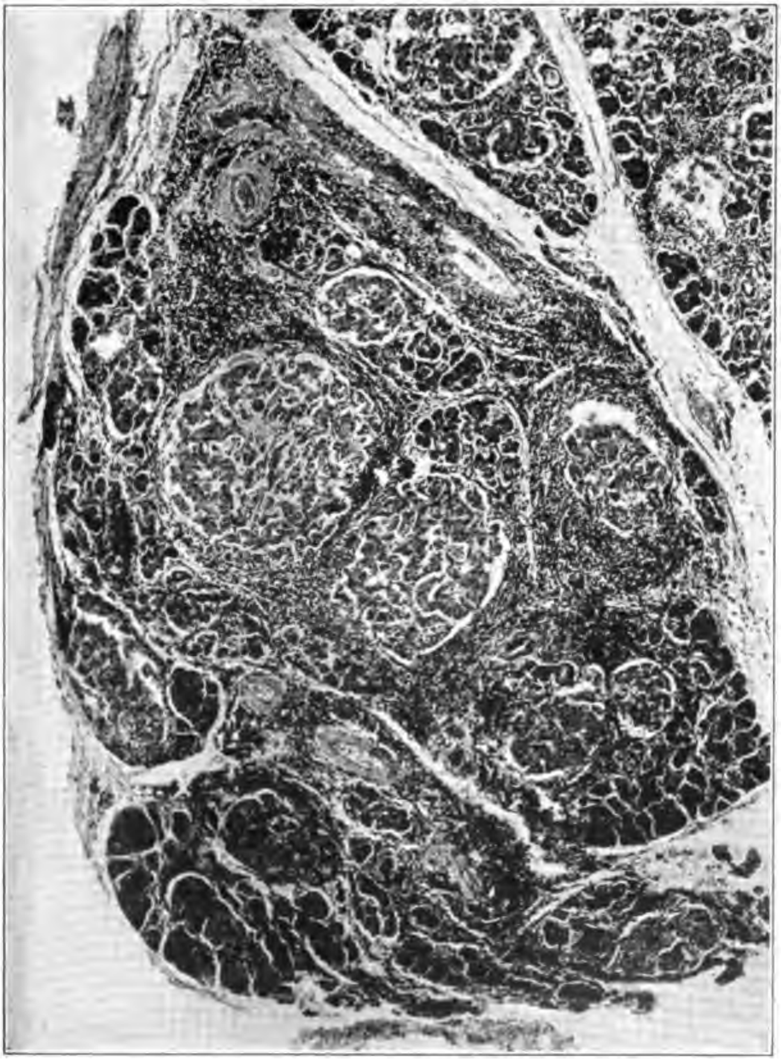

FIG. 1. Low power picture showing islands of large size and eosinophilic infiltration of the surrounding tissues.

capable of passing into encysted form gradually show a diminishing influence on the eosinophiles. If, however, the contents of the cyst gain access to the blood stream either by rupture, or by aspiration and injection, an immediate eosinophilic response results. Of the intestinal parasites, the *Ankylostoma duodenale* stands at the head in its ability to produce an eosinophilic response, values as high as 80 per cent. having been recorded. When it is considered that at the same time there is a marked rise in the total number of leucocytes, it can readily be seen that enormous numbers of new cells have been added to the circulating blood. Malaria in its chronic forms has been known to produce 20 per cent. of eosinophiles.

Certain of the infectious diseases present instances of moderate increases in the number of eosinophiles,—rheumatic fever, scarlet fever, vaccinia, the post-critical period in pneumonia when the eosinophiles which have heretofore been completely absent from the blood reappear. In gonorrhea, especially with the incidence of complications, a moderate increase in the number of eosinophiles has been observed, while extreme values of 25 per cent. have been recorded. Amœbic dysentery has also shown high figures, 47 per cent. having been reported in one case.

In tuberculosis we find the eosinophiles playing a rôle of some prognostic importance for they disappear from the blood in cases of advancing involvement by the tubercle bacillus and reappear only when the reparative processes of the body have succeeded in outstripping the ravages of the disease.

The list of dermatoses with which eosinophilia has been associated is a long and formidable one. It is an almost constant accompaniment of urticaria, eczema, prurigo, iodide and mercury erythemata, and all vesicular and bullous diseases. Psoriasis shows an eosinophilia only inconstantly, but it is invariably present in the dermatitis exfoliativa which often follows psoriasis.

A general eosinophilia is also seen accompanying many of the granulomata and neoplasms. Prominent among these is Hodgkin's disease where figures up to 28 per cent. may be encountered. Curiously enough, higher values may be met with after *x*-ray treatment, percentages up to 68 per cent. having been reported. General sarcomatosis and carcinosis may produce a generalized eosinophilic response even to 45 per cent. in cases of the latter. Lymphosarcoma may also produce a general blood-reaction, but to a less degree.

Local Eosinophilia: The eosinophiles are found widely scattered throughout the body under physiological conditions, both in the fluids of the body and also in the tissues. In the hæmolymph glands they are more numerous than in the ordinary lymph glands. They are said to be absent from the mucous membrane of the œsophagus and stomach but are present in considerable numbers in that of the intestine. A diet mainly of meat or one containing many irritating components is said to increase the number of the eosinophiles in the mucosa. During the digestive periods the number in the spleen increases, while in fasting periods only a few can be found. When present they are found only in the pulp. There are no recorded investigations as to their presence in the salivary glands or the pancreas.

They exist in only moderate numbers in the walls of the respiratory

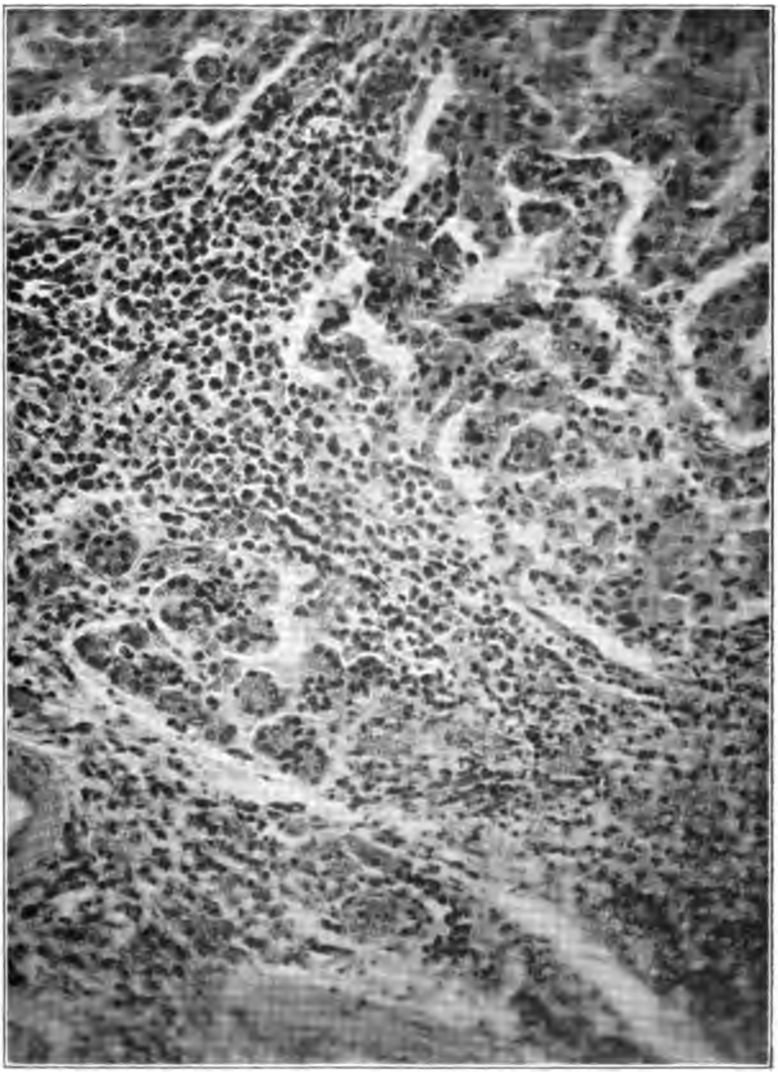

FIG. 2. High power of same showing disordered structure of the islands and the infiltration of the surrounding tissues by eosinophiles.

tract, but are said to present accumulations about the mucous glands of the bronchi.

They may be found in varying numbers in the thymus increasing up to the seventh month and then receding.

In lesions of the gastro-intestinal tract they may be materially increased in number, being found in catarrhal gastritis, atrophic gastritis and especially in tissues showing neoplastic changes. They are supposed to bear a direct relation to the increased mucous secretion of the disordered mucosa rather than to the processes of disintegration. In some forms of appendiceal inflammation they are quite numerous. These accumulations in the mucosa of the intestinal tract are attributed to local immunity reactions comparable to the generalized eosinophilia associated with anaphylactic phenomena.

They are present in the myocardium of cases dying during the course of diphtheria. They are not found in cases proving fatal prior to the seventh day of the disease. Attempts to explain them on the basis of anaphylaxis resulting from the injection of antidiphtheric sera have not been successful, for they have been found as frequently in those who have been and those who have not been treated with serum. They are not present in increasing numbers in comparison with the number of serum injections, and they bear no relationship to the clinical severity of the case. They are found closely gathered about the smaller blood vessels but not in the region of blood extravasations nor near the foci of degeneration.

They are inconstantly present in the pus arising from non-specific inflammations, but in the pus from gonorrheal processes in any site they are abundantly present, increasing in direct relation to the duration of the process until they may make up almost the entire bulk of the suppurative material. In the nasal and bronchial secretions from asthma and hay-fever they are very numerous and may in these instances be largely mononuclear in character. In asthma it has been noted that large numbers of the leucocytes found in the urine may be eosinophiles. The fæces in cases of intestinal helminthiasis may contain large numbers of eosinophilic leucocytes. Pleural exudates often show increasing numbers of eosinophiles as the inflammatory process recedes.

The parasitic lesions of the skin show a local eosinophilia, the cells being arranged either in groups or scattered diffusely through the tissues. Often they are gathered about the smaller blood vessels. In cases of infection with *Trichinella* the eosinophiles are not strictly limited to the immediate vicinity of the parasite, but they may be found throughout the interstitial inflammatory tissue. In lymph node involvement by *Filaria* the eosinophiles may be found in large numbers occupying both the lymph sinuses and the lymph cords. The lymphatics may also be found to contain great numbers of eosinophiles. *Balantidium coli* is also capable of producing a marked eosinophilia, the infected areas of the colon containing many of these cells.

The dermatoses in a similar manner show a local eosinophilia, the tissues surrounding and underlying the lesions often being tightly packed with eosinophiles. The skin diseases capable of producing a local eosinophilia are identical with those which are responsible for the similar generalized blood reaction.

The local eosinophilia about neoplasms is often of considerable importance. This question presents many obscure and puzzling features for no one constant factor can be found exercising any marked influence upon this specialized tissue reaction. One form of tumor may in one site be accompanied by a marked eosinophilia only to find a similar reaction completely lacking in a similar tumor in another region, and one form of tumor in a given region may supply many eosinophiles, whereas a different tumor in the same tissue will show none. In general, however, it may be stated that those tissues which are exposed to the greatest irritation are those in which the eosinophiles are the most numerous.

The carcinomata are fairly regularly accompanied by a moderate eosinophilic infiltration, the uterus being the tissue which is capable of furnishing the greatest numbers of these cells. They are also present to a lesser degree in sarcomata of all types. They are usually infrequent in the hypernephromata. Benign growths may also show an eosinophilia, but less regularly and to a slighter degree, than the malignant growths.

The lesions of periarteritis nodosa show regularly a diffuse admixture of eosinophilic cells.

CASE REPORT

The child whose pancreas is the subject of this paper was born on the 25th of March of this year at the Nursery and Child's Hospital. It was the last of fourteen pregnancies, one of which terminated in a still-birth; all the others however, including this one were normal in all respects. The child was living until labor began, but as it was a very large child, weighing over ten pounds and being a breech presentation with a long difficult labor, extraction was protracted and the child was born dead. There were no external abnormalities, no stigmata of syphilis, and all the organs were found in an approximately normal condition. The pancreas was grossly quite normal. The cause of death was ascertained to be a fracture between the third and fourth cervical vertebrae, consequent upon forcible extraction in the breech position. No suspicion of anything pathological was aroused in the routine microscopical examination except for a slight degree of degeneration in the parenchymal cells of the liver, kidneys and adrenals, until the pancreas was reached, where a most unusual and interesting condition was found. This change affected all the constituents of the pancreas, the islands of Langerhans, the interstitial tissue and last and least the acinar tissue. The same condition existed in all parts of the organ examined.

The islands were enormously increased in size and almost visible to the naked eye. They were much distorted and their usual orderly internal structure was much obscured. They were made up chiefly of two types of cells: (1) A cell which comprised the main bulk of the island and was large with a granular deep staining cytoplasm and an ill-defined cell outline. The nuclei differed much in size, some were very large indeed and presented a clear protoplasm, a definite chromatin network, and in some cases a distinct nucleolus. The cytoplasm of the cells in many areas resembled a syncytium. These cells suggested in many of their properties neoplastic cells. (2) The second type of cell was much less numerous and was arranged principally about the periphery of the islands. It had a small darkly staining nucleus, more or less oval in outline, in which no chromatin network could be made out. Its cell outline was somewhat more definite than that of the preceding cell, and its cytoplasm took a pinker stain. Occasional eosinophiles could be seen in among the cells of the island. In the spaces which surrounded the capillary loops could be seen numbers of beautifully granulated eosinophiles, which were in greater part multilobular, although mononuclear cells were present in fair numbers. In the connective tissue of the capillary loops the eosinophiles seemed to be surrounded by delicate connective tissue strands. The connective tissue of the pancreas presented the most extraordinary eosinophilic infiltration, the entire tissue having been simply converted into eosinophilic tissue, each cell, in many instances, surrounded by delicate connective tissue fibrils. Between the eosinophile cells could be seen occasional connective tissue cells and only very infrequently a polynuclear leucocyte. The tissue was so densely infiltrated with eosinophiles that it was difficult to demonstrate any particular condensation about the blood vessels. The capillaries were engorged and the lining endothelium seemed enlarged and swollen, but only occasional eosinophiles could be found within the capillaries. There was a large amount of free eosin-staining granular material present in the section. Scattered here and there singly and in small clumps between the cells could be seen long needle-like crystals pointed at each end and somewhat thicker at the middle, which took a pink stain, and seemed to be typical Charcot-Leyden crystals. The acinar tissue was much reduced in amount and the acini were in large part isolated from one another by a uniform increase in the amount of inter-acinar connective tissue which appeared oedematous and was loaded with eosinophiles. The majority of the acinar cells appeared quite normal but occasional vacuolated cells and indeed entire degenerating acini could be found. The eosinophile cells did not invade the acini.

Attempts to clear up the etiology of this condition have been without success. Tissue stained by the Levaditi method and examined for spirochaetae has not disclosed any of these organisms. Efforts to find bacteria in preparations stained by the Gram method and with Giemsa's stain have also been unsuccessful.

The question of the origin of the eosinophiles, whether by emigration from the blood vessels or by local production will be left open.

It is equally impossible to give any definite opinion as to the underlying cause for this peculiar picture, but it would seem that it was associated with the islands of Langerhans. The appearance of the cells of the islands indicated that some unusual process was in course there and it is conceivable that some abnormality in their metabolism may be responsible for the eosinophilic infiltration.

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Discussion:

DR. PAPPENHEIMER: I would like to ask the Society for suggestions about this case. It seems to be an unusual condition and none of the long list of possible causes of eosinophilia, which Dr. Riley has cited seems applicable here. In no inflammatory condition with which I am familiar, is the exudate composed entirely of eosinophilic cells. There are, of course, a great many eosinophiles in gonococcus infections of the mucous membranes, but in Dr. Riley's case the exudate is exclusively eosinophilic. Active blood formation in and about the pancreas is not uncommon in new-born infants. In this case, however, the absence of other blood elements and of active hematopoiesis in the liver—and the fact that the eosinophiles are almost all polymorphonuclear makes it certain that the areas are not to be regarded as blood-forming islands.

Dr. Eisenbrey: The only thing I have seen resembling this was in a case of Hodgkin's disease, where the eosinophilic infiltration was very nearly as marked. Occasionally in epithelioma of the cervix you see the exudate composed almost entirely of eosinophiles, but these conditions have no bearing on this particular case.

PATHOLOGICAL FINDINGS IN A CASE OF AMAUROTIC FAMILY IDIOCY

SARA WELT-KAKELS, M.D.

As it is the writer's intention to publish later a paper upon this subject *in extenso,* only a brief abstract of the report will be given on this occasion in connection with the demonstration of sections from a case of amaurotic family idiocy.

A general review of the contributions to the present knowledge of the subject will be omitted at this time and attention will be given to the discussion of the case. Pathological findings were demonstrated by microscopical sections of the brain, spinal cord, and spinal ganglia of a girl who died from this disease when 23 months old. The nervous tissues showed widespread degenerative processes involving the gray nerve cells, consisting of balloon-like swellings of the cell body and dendrites with the disappearance of the Nissl bodies and a peripheral displacement of the nucleus. In more advanced degeneration, the cells appeared like structureless masses. These alterations of the cells were especially well demonstrated in sections stained with Bielschowsky's stain.

Sections of the ductless glands of the patient were also exhibited. The thymus showed marked increase in connective tissue, mainly in the septa. Hassal's bodies appeared to be increased in amount, with disappearance of thymic parenchyma.

The thyroid sections showed no changes.

The adrenals showed slight parenchymatous degeneration; the cortical substance was somewhat shrunken.

The hypophysis showed increase in the number of acidophilic cells.

The examination of the ductless glands seemed to be very opportune, since Dr. Harlow Brooks reported marked alterations in the thymus, adrenals and pituitary glands in a case of amaurotic family idiocy, juvenile type. These lesions suggested to Dr. Brooks atypical functional activity and a possible causal relationship in this disease. The findings in the ductless glands of my case did not seem to justify the assumption that they were either direct or indirect factors in the causations of this fatal malady, nor were the lesions found in the endocrinous glands pathognomic for amaurotic family idiocy, infantile type.

A careful comparative study of ductless glands from children of approximately the same age, which, by courtesy of Dr. A. M Pappenheimer I was given the opportunity to examine at the Nursery and Child's Hospital, seemed to show that similar changes in the endocrinous glands occurred in children who succumbed to other diseases.

Discussion:

Dr. Pappenheimer: Was the sympathetic system investigated?

Dr. Welt-Kakels: No, so far as I know, in none of the cases.

Dr. Levin: Did I understand that some chemical examination had been made of the central nervous system, or not? Was any thing found chemically that would indicate where the trouble lay? Most probably this is a metabolic condition, and not an infection. In this case some abnormal products in the brain may indicate the condition.

Dr. Welt-Kakels: Mott found diminution of sulphur and phosphorus. This was not corroborated by Heiman, Cohn and Bookman.

MORPHOLOGICAL CHANGES INDUCED IN TUMORS UNDER THE INFLUENCE OF ROENTGEN RAYS AND RADIUM

ISAAC LEVIN, M.D.

(From the Department of Cancer Research of the Montefiore Home)

The physical properties of Roentgen and gamma rays emitted by radio-active substances are completely analogous to the rays of light. Both kinds of rays represent, in accordance with the recent physical conceptions, waves of ether. The wave length of actinic rays, i. e., of Roentgen and gamma rays, are a thousand and more times shorter than the shortest ultra-violet rays of light. As a result the rays of light are absorbed completely by the first superficial layers of a substance which they traverse. The actinic rays, on the other hand, penetrate into deeper layers of the substance, and only a fraction of them is lost through absorption by the more superficial layers.

The biological action of the two kinds of rays is also analogous. This analogy is very evident in the so-called *x*-ray burn of the skin. When a large dose of soft *x*-rays is applied to the unprotected skin there takes place an erythema, blistering and ultimately the condition is qualitatively identical with a sunburn and only differs from it in degree. The browning of the skin which takes place as a result of an *x*-ray burn, or occasionally after *x*-raying of the skin even without the formation of a burn is identical with the pigmentation of the skin after a sunburn. Here again the difference is only in degree.

Fr. Bernig in a recent review states that the rays of light, or rather of the ultra-violet light, which is the only biologically active part of the spectrum, produce the following effect on the skin: Direct destruction of the cells; thrombosis of the blood vessels (through the direct influence of the rays on the endothelium and musculature of the vessels) and sero-haemorrhagic inflammation. The latter ends with the formation of hypertrophic connective tissue. It will be seen later that these morphological changes are very similar to the changes induced in tissues by actinic rays.

The analysis of a biological action of any agent is much simpler in the uncomplicated cellular structure of a plant or lower animal than in a vertebrate. A. Richards came to the following conclusions from his studies on the effect of *x*-rays on the rate of cell division in the early cleavage of *Planorbis*. The first effect of exposure to *x*-rays upon the rate of cleavage of the eggs of *Planorbis* is to stimulate mitotic activity. Following the phase of acceleration a phase of depression sets in; the end result is a marked retardation in the development of the egg.

In a vertebrate the actinic rays produce a biological effect on all the tissues of the organism, but the degree of injury varies greatly with the different tissues. It may be stated as a general rule that tissues consisting of less differentiated, younger cells also cells in a state of active proliferation are most deeply influenced by the rays. It is probable that in these complex vertebrate tissues the first effect of the rays also consists in an acceleration of the cellular functions. But it is very difficult to demonstrate such a phenomenon morphologically. Histologically one sees finally complete necrosis of the cell. Every tumor consists of undifferentiated young cells in a state of active proliferation, consequently a strong selective biological influence of the rays on tumors must be considered certain *a priori*. The morphological changes which take place in tumors under the influence of the rays differ with the different types of tumor and it may be well to consider them separately.

Carcinoma.—The first morphological changes which occur in carcinomatous tissue under the influence of actinic rays are observed in the tumor cells themselves and are manifested by the vacuolization of the protoplasm, pycnosis of the nuclei, karyolysis and complete necrosis of the cell. All this is accompanied by a round cell infiltration which replaces the destroyed carcinomatous cells. Somewhat later there begins formation of dense sclerotic connective tissue poor in blood vessels. This connective tissue formation may become very extensive, surround islands of carcinomatous cells and assist in the destruction of the latter. The following case of carcinoma of the sigmoid with metastatic dissemination in the peritoneum which came to autopsy after six months of continuous *x*-ray treatment demonstrates the importance and extent of this connective tissue formation.

The patient died of acute intestinal obstruction. At the autopsy there were found in the peritoneal cavity several loops of the intestines adherent by old adhesions to the posterior surface of tumor mass in the sigmoid. The peritoneum, especially in the pelvis, was studded with numerous white plaques, varying in size from one mm. to one-half cm. in diameter—the larger ones were quite firm to the touch. On section the mesenteric lymph nodes were rather small and reddish in color.

Microscopical examination of a section taken through two loops of the small intestine which were firmly bound together by old adhesions, showed that the adhesions consisted of a thick layer of connective tissue containing occasional nests of tumor cells. These nests were rather more abundant, however, in the muscular layers of the intestine than in the fibrous tissue connecting the loops. The tumor cells stained very deeply.

Another section taken from the edge of the colostomy wound showed a bit of skin with a wide zone of connective tissue beneath in which there were occasional groups of tumor cells in the muscular layers and mucous membrane of the intestine. There were relatively few groups of tumor cells in the muscular layers, but the mucous membrane was rather densely infiltrated with tumor. In the muscle, especially, one could see groups of tumor cells, apparently degenerated, in the midst of a mucoid material of the character that is found in the so-called colloid carcinomata.

The peritoneal nodules were composed of dense connective tissue, with occasional groups of tumor cells, which, as elsewhere, stained poorly. No follicles of colloid material were present. In some nodules no tumor cells were found.

Sarcoma.—The morphological changes induced in sarcomatous tissue by actinic rays is identical with those induced in carcinoma. The following case of a giant cell sarcoma of the lower jaw illustrates this effect. A small piece was removed for microscopic examination which showed a sarcoma with numerous giant cells and actively growing spindle cells. Following eight weeks of combined radium and *x*-ray treatment another section was taken from the same region from which the previous one was obtained. The section showed a very loose connective tissue, relatively poor in cells and beneath this a denser zone of cellular connective tissue. No giant cells or distinct spindle cells were present. A radiogram taken at the same time showed that the whole tumor was surrounded by a great deal of newly formed bone. Examination through the mouth showed the tumor to have completely disappeared and there was present instead a large cavity lined by a shell of bone.

Rhinoscleroma.—Infectious granulomata, whether tuberculous, syphilitic or of any other kind, are influenced by the actinic rays as lympho-sarcomata. The lymphoid cells are destroyed and replaced by dense sclerotic fibrous connective tissue. Rhinoscleroma is an infectious granuloma, characterized by the presence in the granulation tissue of the so-called Mikulicz cells. The latter are degenerated lymphoid cells, enlarged in size, inside of which may be found frequently the Frisch bacillus, the etiological factor. Under the influence of radium the observed cases showed a disappearance of the obstructing swelling of the naso-pharynx. The microscopic examination showed that the granulation tissue was completely replaced by dense connective tissue.

CONCLUSION

Thus the most generally observed morphological change in tumor tissue under the influence of radiation is the extensive formation of sclerotic connective tissue. Some observers maintain that this new connective tissue formation is the only direct effect of radiation. The destruction of the tumor cells is secondary and is due to lack of nutrition. This opinion cannot be accepted as true. In the first place, as was shown above, the first change noted in carcinoma was the destruction of the cells and only subsequently did the connective tissue form. Moreover, in certain conditions, for instance rodent ulcer of the skin, the epithelioma heals, is covered with skin epithelium and there is no formation of connective tissue.

Other investigators assert that the destruction of the tumor cells is the only direct affect of radiation. The formation of connective tissue, in accordance with this view is secondary to the accumulation of dead tumor cells and is analogous to formation of connective tissue about foreign bodies. This assumption is also hardly tenable. In the first place the amount of connective tissue formation in the peritoneal nodules of the carcinoma of the sigmoid reported above was entirely out of proportion to the number of carcinomatous cells present. Moreover, if such young connective tissue were formed only by the stimulus of dead tumor cells, then the actinic rays subsequently would dissolve this connective tissue as easily as it dissolves a keloid. But this does not take place and the amount of connective tissue usually increases with subsequent radiations.

It must be concluded then that morphological changes which take place in tumor tissue under the influence of actinic rays are two-fold. There occurs an inhibition and ultimate destruction of the tumor cells with irritation and consequent proliferation of surrounding connective tissue. But the beginning of this new connective tissue cannot be looked for in the normal tissue surrounding the tumor.. The post-mortem study of the carcinoma of the sigmoid showed that there was no new connective tissue formed anywhere in the normal organs under the influence of radiation. The source of this new connective tissue must be looked for either in the stroma of the tumor or in the round cell infiltration which always closely follows the destruction of the tumor cells by the rays.

It may be stated then that the destruction of the tumor cells is the primary and the formation of new sclerotic connective tissue a secondary but at least as important a phase in the morphological changes which take place in tumors under the influence of actinic rays.

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Discussion:

DR. SITTENFIELD: I would like to ask Dr. Levin whether he has studied vascular changes in the tumor and whether he has any notion as to just how the cells are influenced,—whether by dissociation of the chemical component parts of the cells or by destruction of the generative apparatus of the cell as held by Wassermann, or by inactivation of intracellular ferments?

DR. LEVIN: So far as blood vessels are concerned, there takes place a condition similar to endarteritis obliterans which decreases the amount of blood supplied to the tumor causing inhibition and death of the tumor cell. As to Dr. Sittenfield's second question, it is impossible at present to determine the mechanism of the biological action of the actinic rays.

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AMYLOIDOSIS: (*a*) INCIDENCE. (*b*) REPORT OF CASE WITH EXTENSIVE AMYLOID-LIKE DEPOSITS IN ORGANS NOT SHOWING ANY OF THE CHARACTERISTIC REACTIONS

R. A. LAMBERT, M.D.

(From the Pathological Laboratory of Montefiore Hospital and the Department of Pathology of the College of Physicians and Surgeons)

(*a*) INCIDENCE

Among fifty-five autopsies performed at Montefiore Hospital during the past eight months nine cases of amyloidosis have been encountered, an incidence of 18 per cent. Chronic pulmonary tuberculosis was the cause of death in twenty-one cases, eight of which, or 38.1 per cent., showed a deposition of amyloid in various organs.

As will be seen from the accompanying table these figures suggest that amyloidosis is far more common in this hospital than in other institutions from which statistics on this point are available. Fortunately, we are able to compare these figures with those obtained from the records of the Presbyterian Hospital where during the past seven years only six cases of amyloidosis have been observed in approximately eight hundred and fifty autopsies, an incidence of 0.7 per cent.

Also at the College of Physicians and Surgeons, where autopsy material is received from various hospitals, only twelve cases of amyloidosis have been seen in nearly one thousand autopsies. Statistics from foreign hospitals show, too, a far lower incidence. It has seemed to us that a consideration of the possible causes for this striking difference in frequency should prove of interest.

TABLE

	Chronic Tuberculosis	Cases Showing Amyloidosis	Incidence, Per Cent.	Non-Tuberculous Cases	Cases Showing Amyloidosis	Incidence, Per Cent.	Total Autopsies	Total Number Cases of Amyloidosis	Incidence, Per Cent.
Montefiore Hospital.....	21	8	38.1	34	1	3.0	55	9	16.4
Presbyterian Hospital . .	50	2	4.0	800	4	0.5	850	6	0.7
College of Physicians and Surgeons	158	9	5.7	842	3	0.35	1,000	12	1.2
Vienna (Blum's statistics)	2,500	221	8.8	15,653	76	0.49	18,153	297	1.5
Kiel (Wicht's statistics).							2700	97	3.6

It should be stated in the beginning that the number of autopsies in the Montefiore series (fifty-five) is rather small for any conclusive statistical study, but the fact that only sixteen[1] cases of amyloidosis have been found in one thousand, eight hundred and fifty autopsies outside of Montefiore Hospital goes far to prove that the high incidence there cannot be regarded as accidental.

In discussing this problem with Dr. Wachsmann, Superintendent of Montefiore Hospital, and others, it has been suggested that the patients in that institution differ from those in other general hospitals in at least three respects: (1) Most of the patients are foreign born. This was true of all of the cases of amyloidosis;

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[1] Eighteen cases are recorded in the table but two of these were Montefiore cases, the material from which was sent to the College of Physicians and Surgeons.

(2) All patients belong to the Jewish race; (3) The tuberculous patients admitted differ in certain respects from those in many other hospitals.

Regarding the first factor, namely, the nationality of the patient, it has been stated by many who have attended autopsies in Berlin and Vienna, that amyloidosis is much more common in Europe than in America. The statistics from Vienna and Kiel (see table) seem to disprove this idea, since the percentage of amyloidosis in total number of autopsies as well as in cases where death was due to tuberculosis is not much higher than at the College of Physicians and Surgeons, and not nearly so high as in the Montefiore series.

At Vienna in calculating the percentage of tuberculous cases showing amyloid deposits, cases of acute general miliary tuberculosis and tuberculous pneumonia were probably included, whereas such cases were carefully omitted in the preparation of our statistics. We made this selection of tuberculous cases, firstly, because amyloid is practically never found in acute tuberculosis; and, secondly, because all the cases at Montefiore Hospital were chronic. Therefore a fair comparison necessitated a selection of cases of the same type at the other institutions. If we should include in our statistics from the College of Physicians and Surgeons and the Presbyterian Hospital deaths from acute tuberculosis the percentages of amyloidosis in tuberculosis would fall considerably lower than those for Vienna and Kiel.

Looking into the question of nationality of the six cases of amyloidosis at the Presbyterian Hospital, we find one Italian, one Englishman, two Hungarians, one American of German parentage, and one American negro. The percentage of foreign-born patients admitted to the hospital is about 40 per cent., but if to this group are added those of foreign parentage the percentage of "foreign" patients would probably reach 75. It is seen therefore that these figures do not give much support to the idea that foreigners are very much more susceptible to amyloid disease.

The second suggestion, that of a racial predisposition, is one upon which our available statistics are not altogether conclusive. At the College of Physicians and Surgeons no record of race has been kept. At the Presbyterian Hospital, although about 12 per cent. of the admissions are Hebrews, autopsies upon people of

Jewish faith are rare, comprising less than 5 per cent. of the total number. Thus we are able to compare the records of a hospital where all are Hebrews with those of another where there is only a small percentage of such cases. This comparison (see table), which shows at Montefiore Hospital an incidence of amyloidosis nine times as great in cases of chronic tuberculosis and six times as great in non-tuberculous cases as at the Presbyterian Hospital, certainly suggests a definite racial susceptibility. However we hesitate to form any conclusion on this point because a careful study of the cases at the two institutions, which brings us to the third possible predisposing factor, shows that the types of patients, especially those of chronic tuberculosis, admitted to the two institutions differ very materially.

The patients coming to autopsy from the tuberculosis division of Montefiore Hospital show as a rule far advanced pulmonary tuberculosis of long duration (average of those coming to autopsy, three years and four months). At the Presbyterian Hospital the cases of chronic pulmonary tuberculosis give a history usually of short duration (average of those coming to autopsy, nine months). While we have included in the fifty Presbyterian Hospital cases only those which were obviously chronic with cavity formation in lungs, or extensive bone lesions, we do not think that the comparison between these and the Montefiore cases is altogether satisfactory.

In order to settle conclusively the question of a racial susceptibility it will be necessary to obtain statistics from an institution receiving non-Jewish patients, more nearly of the type found at Montefiore Hospital.

The experimental work of Davidsohn and others, who have shown that certain strains of mice and rabbits are much more susceptible than others to the development of amyloidosis, is at least suggestive in this connection.

The tentative conclusions from this brief review of twenty-seven cases of amyloidosis seen in New York among nineteen hundred and five autopsies are as follows:

1. The influence of nationality upon the incidence of amy-

loidosis is negligible. That the condition is less common in America than in Europe is doubtful.

2. A predisposition to amyloid disease on the part of Hebrews is suggested, though not proved, by the high incidence observed at Montefiore Hospital.

3. The most important factor in determining the incidence of amyloidosis in any hospital is the type of patient admitted. Tuberculosis of long standing, involving extensive tissue destruction, is the condition most often associated with amyloidosis (70 per cent. in our cases).

(*b*) REPORT OF CASE WITH AMYLOID-LIKE DEPOSITS IN ORGANS

In eight of the nine cases of amyloidosis observed at Montefiore Hospital, and in all of the eighteen cases at the Presbyterian Hospital and the College of Physicians and Surgeons, the amyloid-containing organs showed the usual reaction with iodine. In one of the Montefiore cases, however, the reaction was entirely negative.

The case was that of a young man, twenty years old, who had shown symptoms of pulmonary tuberculosis for two years: cough, expectoration, hemoptysis, afternoon fever, etc.

At *autopsy* lungs showed extensive tuberculous lesions with numerous cavities in right lung. Spleen weighed 800 grams, was hard and on section presented the picture of a typical "sago" organ. The liver weighed 2,200 grams, was very firm and friable and showed the characteristic bacon-like translucency. In the kidneys no striking gross changes were apparent. The adrenals were rigid as though preserved in formalin.

Microscopical Examination: In the spleen, liver, adrenals and glomeruli of kidneys an extensive deposition of a hyaline pink-staining material was found, which presented the distribution characteristic of amyloid.

Application of iodine to the gross specimen, as stated above, gave no reaction. Negative results were likewise obtained with methyl-green and iodine-green. Methyl-violet gave in places a very faint reddish tinge which appeared to be more pronounced in the hyaline material in immediate proximity to the blood vessels. This faint staining was clearly seen in the liver sections. The tests were applied on fresh tissue and on specimens fixed for a short time in formalin. Some of the sections were first treated with weak acetic acid in order to neutralize any alkali present.

There seems little doubt, therefore, that we are concerned here either with deposits of an incomplete amyloid material or a general hyalinosis, a condition not recognized by those who have worked in this field. It has been clearly demonstrated by Davidsohn and others in the experimental amyloidosis of mice and rabbits that there are at least two stages in the development of amyloid. In the earlier stage the deposits do not give the usual reaction. The reaction with the methyl-violet group of dyes appears first, usually in the spleen. The iodine reaction is obtained later. Only the completely finished product, which is found rarely in organs other than the spleen and liver, gives the iodine-sulphuric acid reaction. In the present case such an extensive deposition would suggest a late rather than an early stage. It is possible, however, that there may have been a very rapid formation and deposition of the incomplete amyloid during the last few weeks of the patient's life, and that sufficient time had not elapsed for its complete development. So far as we have been able to find out no case like this one, with such extensive deposits giving none of the usual reactions, has been described.

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Discussion:

Dr. Field: One reason that would suggest itself to me why we see so few cases of amyloidosis is that most of our material is obtained from hospitals which have an "acute" service. I imagine that more is seen on the Island in the almshouse and among their chronic cases than in Bellevue where the service is "acute." This has impressed itself on me since going down to the Neponsit Hospital, where children with bone and joint tuberculosis are treated. The number of cases in which the clinicians have diagnosed amyloidosis is comparatively large. In Louisville I saw, in my two years there, more amyloidosis than I have seen in my twelve years in New York. The cases were found among negroes who had had chronic suppurative lesions extending over periods of many years. I do not think that the suppuration is limited to any one type of organism. The only constant feature in my experience has been a suppurative process, though, of course, this generally showed many types of microorganisms.

DR. ZINSSER: I would like to ask whether amyloid change has any relation to any particular type of suppuration. Is it associated with any specific variety of microorganisms or is it the result of suppuration in general?

DR. LARKIN: We must ask what is amyloid degeneration. Chemists have not given us definite information as regards its chemical content. We are not in a position to tell the different stages of amyloid or hyaline degeneration. Neither macroscopical nor microchemical tests are absolutely proved. I would be chary of putting down such a specimen as amyloid, where the microchemical test does not confirm it. I think Dr. Zinsser's question is a pertinent one as to what are the factors that produce it. We look for amyloid degeneration in chronic suppurative conditions, bone tuberculosis, psoas abscess, and so on. There are a number of suppurative conditions of which we do not know the character, which do not produce amyloid. We must differentiate these cases from those giving the microchemical test. This specimen does not represent amyloid conditions *per se*. It is one of the intermediate groups.

DR. LAMBERT: Replying first to Dr. Zinsser's question: in the experimental production of amyloid it has been shown that almost any pyogenic organism may be used. Killed cultures are quite efficacious.

Regarding Dr. Field's last statement, I think that the common association of amyloid disease with tuberculosis is too well established to warrant discussion. In Blum's series at Vienna (two hundred and ninety-seven cases in eighteen thousand one hundred and fifty-three autopsies) tuberculosis was the etiologic factor in 79 per cent.: in our series, 70 per cent.

With Dr. Field's other remarks I am in thorough accord. His experience makes me feel certain that the high incidence at Montefiore Hospital is probably to be attributed to the very chronic type of tuberculosis treated there.

REPORT ON TWO FORMS OF CARDIAC ABNORMALITY

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(*From the Department of Pathology of the College of Physicians and Surgeons and the New York Nursery and Child's Hospital*)

The study of the two hearts which form the basis of this report is presented more with the idea of placing the results on record than of giving in connection with it any extended discussion of the anatomical or pathological features of the organs. The material was obtained from the Pathological Laboratory of the New York Nursery and Child's Hospital. During the past year we have met with almost all the common types of cardiac abnormality and in addition we have found three conditions of more than usual interest:

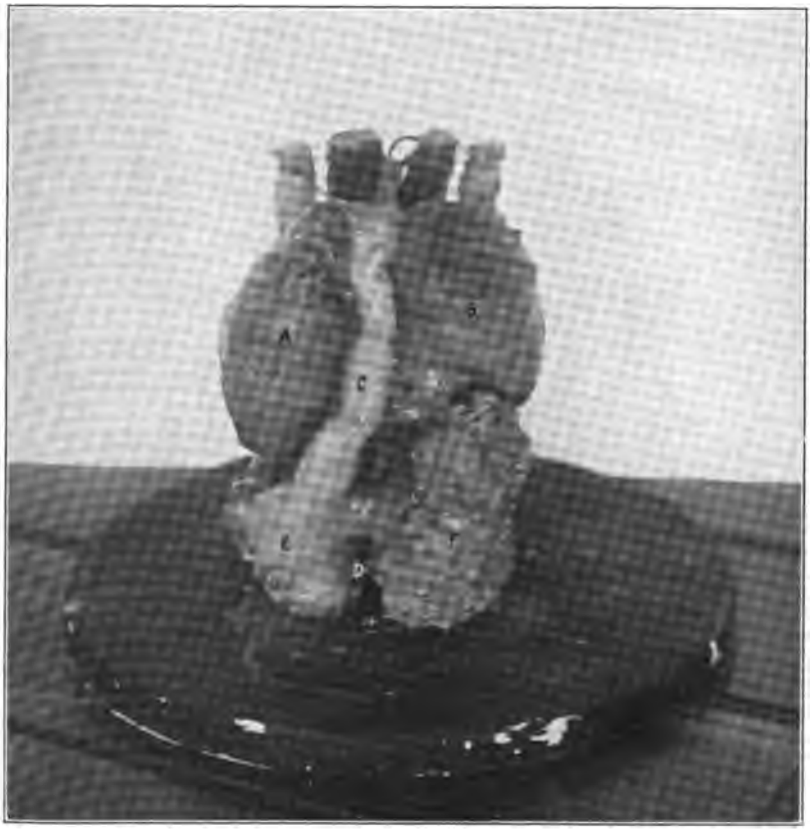
(*a*) Complete transposition of the arterial trunks.
(*b*) Complete absence of the interventricular septum.
(*c*) Aortic atresia and rudimentary left ventricle.

The first two will be shown at this meeting; the third will be made the subject of a separate report at some later date.

COMPLETE TRANSPOSITION OF THE ARTERIAL TRUNKS

CASE I. *Pathology.*—In this case, we find the ventricular portion of the heart to be normal in all its features; position, size, and composition of its costo-chondral surface. The heart is normally divided by its interventricular groove. The right ventricle externally appears somewhat larger than the left and forms the apex of the heart, but upon opening the organ, we find that the cavity of the left ventricle is larger than that of the right. The walls of the right ventricle are thicker than those of the left. The auriculo-ventricular valves are thin, delicate, and present extra flaps; the right auriculo-ventricular orifice being guarded by a valve which presents four flaps, the left auriculo-ventricular orifice by a valve with three flaps. The auricles are normally formed and separated from one another and there are no defects in the interauricular septum. The foramen ovale is still patent along its anterior border.

The great vessels present a condition of complete transposition, the aorta arising from the right ventricle, the pulmonary artery from the left. They emerge from the heart in an almost directly transverse plane close together and near the anterior aspect of the heart. They both pass upward and somewhat backward. The aorta then sweeps to the left and posteriorly over the bifurcation of the pulmonary artery, giving off its three great vessels from its superior aspect. The inferior aspect of the vessel receives the ductus arteriosus which is comparatively long, twisted, and patent. The aorta then passes over the root of the left lung. The pulmonary artery passes upward and backward under cover of the aorta and divides into three vessels, the ductus arteriosus, which arises slightly to the left of the bifurcation, and the two pulmonary arteries. The coronary arteries are derived from the



Photographs of reconstructions of the cardiac apparatus in three cat embryos—7, 9, 11 mm.—in length, showing the effect of torsion on the truncus arteriosus and the division of that vessel into the aorta and the pulmonary artery.

FIG. 1. 7 mm. cat embryo. *a,* right auricle; *b,* left auricle; *c,* common arterial trunk; *d,* interventricular septum; *e,* right ventricle; *f,* left ventricle.

aorta. The semilunar valves are normal both in number and arrangement as are also the pulmonary veins.

In this case we have an arrest of torsion of the great vessels whereby the orifice of the aorta remains on the right side, that of the pulmonary artery on the left. The interventricular septum, rising from below and coming in contact with the septum trunci, establishes connection between the aorta and the right ventricle, and the pulmonary artery and the left ventricle. The difficult thing to reconcile with this course of events is that in this case

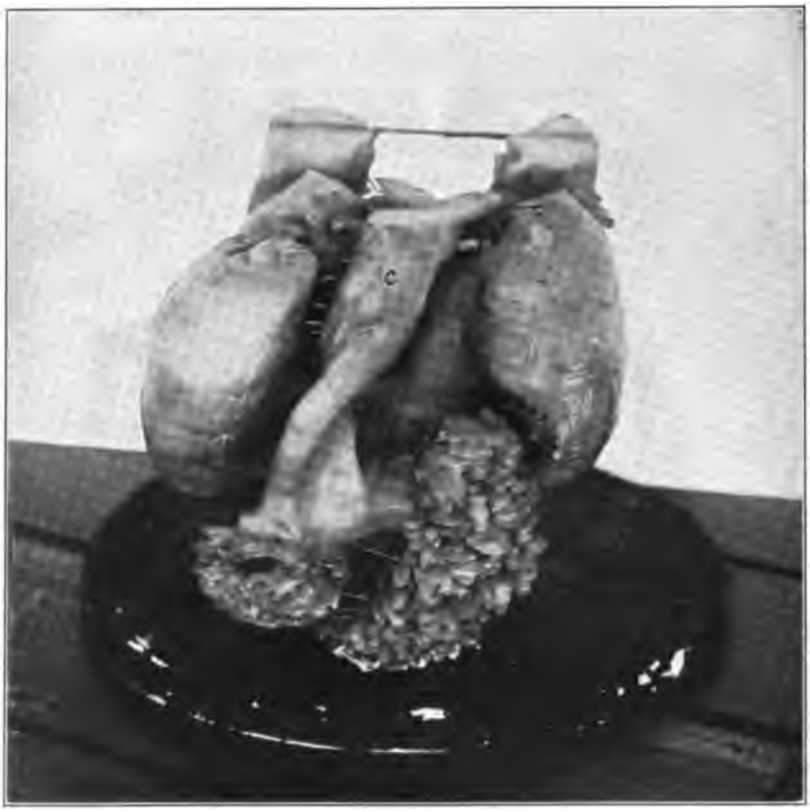


Fig. 2. 9 mm. cat embryo. *c*, common arterial trunk. The truncus shows here the effect of the torsion.

the ventricles have obeyed the tendency to rotate and have taken up their normal position, whereas the truncus which is supposed to obey the same factors in its rotation has completely ignored these factors and has remained unrotated.

Transposition of the Arterial Trunks.—According to the teachings of Rokitansky, transposition of the great vessels, *Rechtslage* of the aorta, as well as certain types of developmental stenosis of the aorta and pulmonary artery, are due to irregularities in the formation of the septum within the aortic bulb and to its malunion with the interventricular septum.

Types.—(*a*) Complete transposition in which the vessels arise from the ventricle to which they do not normally belong.

(*b*) Corrected transposition in which the vessels arise from the proper ventricle but present an inverted relationship to each other.

(*c*) Partial transposition.

In studying these conditions it is necessary for us to keep in mind the processes through which the heart passes in its development in order that we may understand the anomalies which arise through arrest of development or perverted development. In order that these factors may be clear in our minds, it may be well for us to review the steps in the development of these structures.

As will be remembered, the primitive heart tube in its growth becomes twisted upon itself in the shape of the letter S, the venous end being situated posteriorly and to the left in the position where the auricles will develop; in front of the venous end of the heart tube lies the ventricular portion and passing upward mesially and cephalad from the ventricular portion lies the common arterial trunk. There are two fixed points to be considered in order that we may understand the process of torsion which is to determine the picture in the truncus; the first, where the venous end of the heart enters the pericardial sac; the second, where the arterial trunks leave the pericardial sac. Continued growth within the pericardial sac along the direction already initiated by the S-shaped tube produces a torsion of 180 degrees of the common truncus by which the relationship of the adult aorta and the pulmonary artery is produced; this torsion is from the left to the right. By the time that this torsion has begun to make itself felt, the process of separation between the aorta and pulmonary artery has been initiated. This separation is produced by the appearance within the cavity of the truncus of two sets of ridges which according to their relationship with the ventricle, are called proximal and distal. The proximal set of two may be called ridges *A* and *B*, and a distal set of four, ridges 1, 2, 3 and 4. The result of the external forces of rotation or torsion on these ridges is the assumption by them of a spiral twist as they pass toward the heart along the interior of the tube. This spiral twist takes a clockwise direction from above downward and to the right; on the exterior of the tube can be found furrows which correspond to the internal ridges.

We will now follow in some detail the origin of the two sets of ridges and their behavior in dividing the tube into the two large vessels. The ridges arise on sides of the tube opposite one another; the ridges *A* and *B* in the lower half, the ridges 1, 2, 3 and 4 in the upper half, of the truncus. They are so arranged that as they spiral downward in a clockwise direction one ridge of the proximal set meets and fuses with one ridge of the distal set on either side of the tube. We have thus in the composition of each ridge three parts; one horn of the septum aorto-pulmonalis (will be described in detail later); one of the ridges 1 and 3 and one of the ridges *A* and *B*. Ridges 1 and 3 are large, 2 and 4 are small.

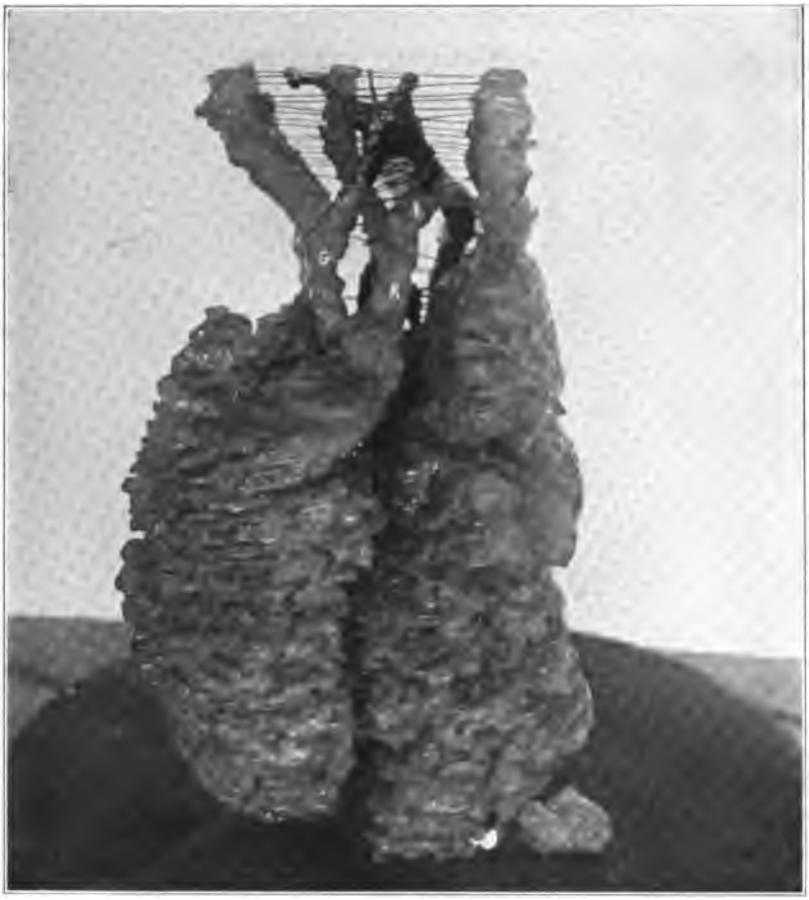


FIG. 3. 11 mm. cat embryo. *g*, aorta; *k*, pulmonary artery; *i*, ductus arteriosus. Complete separation of the truncus into the aorta and the pulmonary artery.

Prior to this there has been a separation of the fourth and sixth aortic arches external to the pericardial sac into what will later become the adult aorta and pulmonary artery. This so-called septum aorto-pulmonalis now invades the portion of the truncus which lies within the pericardial sac and adds itself to the factors producing a separation between the two vessels. We now have the two sets of ridges, *A* and *B*, and 1, 2, 3 and 4, and the septum aorto-pulmonalis. This septum pushes forward its two horns which thus enclose between them a free sickle-shaped edge and finally effects a junction with ridges 1 and 3, which in turn becomes continuous with ridges *A* and *B*. The process thus initiated progresses slowly, the sickle-shaped free margin passes caudally. The ridges become thicker and thicker until they fuse from above downward and the two vessels become completely separated. Through the spiral arrangement of the ridges we now have two curving tubes which looked at from above present a pulmonary artery passing forward and to the right, and an aorta passing posteriorly and to the left closely applied and wrapped about one another.

At a somewhat later time the vessels become independent through their separation by the external furrows which corresponded to the internal ridges. Having now seen the single trunk divided into two separate vessels, we can follow the development of the semilunar valves. Through the fusion of ridges 1 and 3 each vessel contains one half of each fused ridge and in addition the smaller ridge which lies between the larger ridges. The aorta contains ridge 2, the pulmonary artery ridge 4; these smaller ridges fade away distally but in the region of the junction with the ventricle they enlarge and become hollowed out, forming one of the semilunar valves; the other two valves are formed through similar processes taking place in the halves of ridges 1 and 3 left in each vessel.

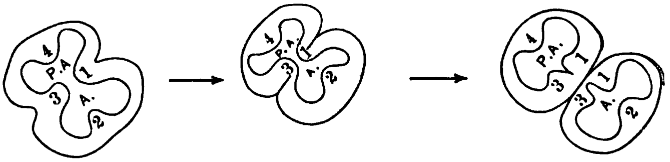
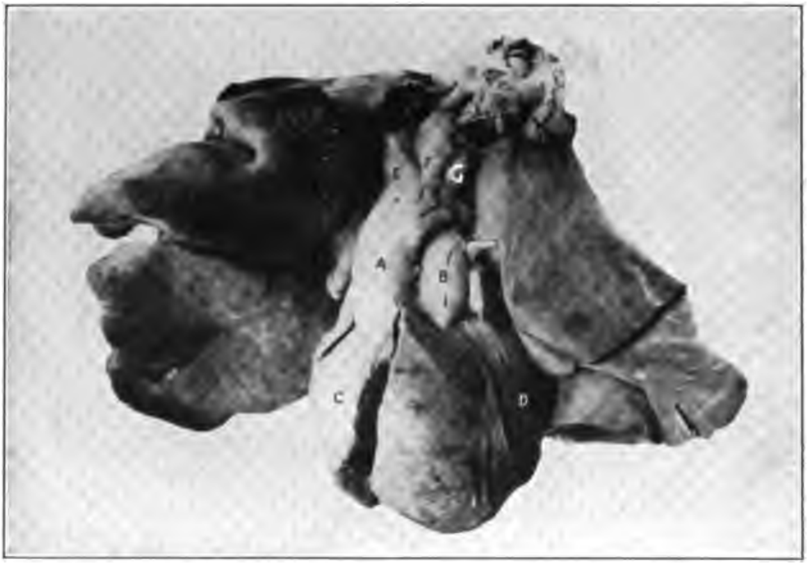


Diagram to illustrate the formation of the semilunar valves which develop by the hollowing out of the lowermost extremities of the ridges 1, 2, 3 and 4. The diagram also illustrates how ridges 1 and 3 participate in the formation of valve-flaps in both the aorta and pulmonary artery.

The two vessels now open into the ventricle which has become divided by the interventricular septum into the smaller right and the larger left half. This septum grows up from the floor and both anterior and posterior walls at the same time so that it presents two horns, an anterior and a posterior, containing between them a free sickle-shaped border. Meanwhile, the most inferior portions of ridges *A* and *B* have grown forward, joined, and have produced what we call the proximal bulb septum. Viewing this from above, we find that it passes from the right superiorly and posteriorly toward the left anteriorly and inferiorly, and thus sets itself in a warped fashion on the interventricular septum which is growing upward to fuse with it.

The anterior cornu of the septum trunci fuses with the anterior horn of the interventricular septum, while the posterior cornu of the septum trunci spreads out on the posterior ventricular wall and fuses with the right extremity of the posterior endocardial cushion of the atrio-ventricular canal. If we follow the boundaries of the interventricular foramen, we now find them as follows: starting from above and behind we have the warped under surface of the septum trunci leading to the anterior cornu of the interventricular septum, advancing downward along the anterior wall to the inferior sickle-shaped intercornual margin, back and finally upward along the posterior cornu of the septum to the right extremity of the posterior endocardial cushion. This space is gradually closed in by downgrowth of the septum trunci and upgrowth of the interventricular septum until they meet and fuse.



Photographs of the specimens embodied in the report.

FIG. 4. Complete transposition of the arterial trunks. *a*, aorta; *b*, pulmonary artery; *c*, right ventricle; *d*, left ventricle; *e*, innominate artery; *f*, carotid artery left; *g*, left subclavian.

As a result of the direction of the septum trunci toward the right end of the posterior endocardial cushion, we find the right atrio-ventricular opening close to the septum, the left separated from it by some distance.

Having seen the mechanism by which the great vessels become separated from one another, it is a comparatively easy matter to see how readily anomalies of the arrangement of the great vessels may occur. Rokitansky, in his schema of the rotation of the great vessels, distinguishes two main groups according to whether the transposition was corrected or not with eight subdivisions in each group, the subdivisions being stages in the spiral twist and the form that the transposition would take in each subdivision were the spiral arrested at that point. In his schema *A* we find the transposition is corrected, for, in spite of the altered relation between the two great trunks, they are connected with the proper ventricle by a compensatory twist of the interventricular septum. In schema *B* we find, however, a true transposition of the trunks, there having been no correction and the trunks being connected with the wrong ventricle. The behavior of the interventricular septum determines whether correction takes place or not.

Partial Transposition.—This is relatively infrequent, but it exists and results in the large vessels arising from the same or a common ventricle and is due to the application of the interventricular septum to either one side or the other of the large vessels instead of between them. In such cases it is the rule to find the orifice of one or the other of the vessels encroached upon.

An anomalous septum may cut off an abnormal cavity as an origin for the two vessels. Unless some other anomaly is present and persists, complete transposition is incompatible with life, for we have the lesser circulation cut off and transformed into a closed circulation. This deprives the blood of any opportunity to gain access to the greater circulation unless it is by some abnormal channel as a patent ductus, a patent foramen ovale or a defect in the interventricular septum; correspondingly, the blood in the greater circulation fails to gain access to the lungs.

Defects of the Interventricular Septum.—As is usually the case with cardiac lesions, we often find other lesions associated with defects in the interventricular septum, the great vessels being often affected. This is readily understandable when we consider the close relationship between the two sets of structures.

Types: (*a*) Complete.
(*b*) Incomplete.

If complete we may find either a condition known as cor triloculare biatriatum or cor biloculare, depending upon the presence or absence of the interauricular septum. In association with the latter we may find a common auriculo-ventricular orifice and an undivided truncus.

These defects may be viewed mainly as arrests of development and in the majority of cases may be explained by a simple schema of the development of the interventricular septum. The septum develops from four component parts:

(*a*) The proximal septum trunci.
(*b*) The anterior horn of the interventricular septum.
(*c*) The posterior horn of the interventricular septum.
(*d*) The intercornual septum.

(*a*) Defects in the septum due to faulty development of the septum trunci constitute the most frequent type. The defect is located close under the orifices of the great vessels and occupies the area known as the "undefended space" or the pars membranacea.

(*b*) Defects due to the failure to develop of the anterior horn of the interventricular septum are located in front of the pars membranacea.

(*c*) Defects due to failure to develop in the posterior horn are correspondingly located behind the pars membranacea.

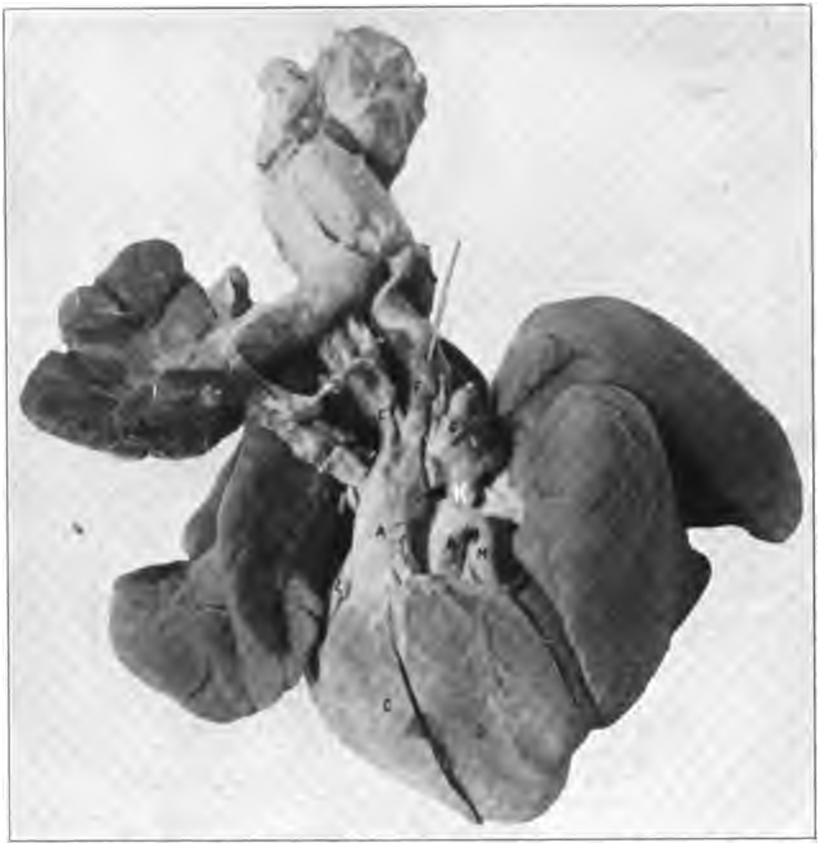


FIG. 5. Complete transposition of the arterial trunks. *a*, aorta; *b*, pulmonary artery; *c*, right ventricle; *d*, left ventricle; *e*, innominate artery; *f*, left carotid artery; *g*, left subclavian; *k*, ductus arteriosus; *i*, thymus; *l*, right auricle; *m*, left auricle.

(*d*) Defects due to faulty development of the intercornual portion may lie at any point in the septum, but they are more usually close up under the pars membranacea and are due to failure of the two septa to join.

COMPLETE ABSENCE OF THE INTERVENTRICULAR SEPTUM

Case II. *Pathology.*—Externally the heart is quite normal in shape. It weighs 170 grams, measures 8 × 7.5 × 6 cm., and has a fairly well defined and formed apex. There is, however, no interventricular groove to be seen and no indication of any division into two ventricles. The great vessels appear to arise from the heart on almost the same plane; the aorta from the right side, the pulmonary artery from the left and slightly posterior so that we have here also a case of transposition of the vessels. The vessels are cut off so close to the heart that their further course cannot be followed but so far as we can see there is no indication of any ductus. Upon opening the auricles, we find that the right auricle is the larger, the right auriculo-ventricular orifice measuring 5 cm. in circumference; the left auricle is smaller, its auriculo-

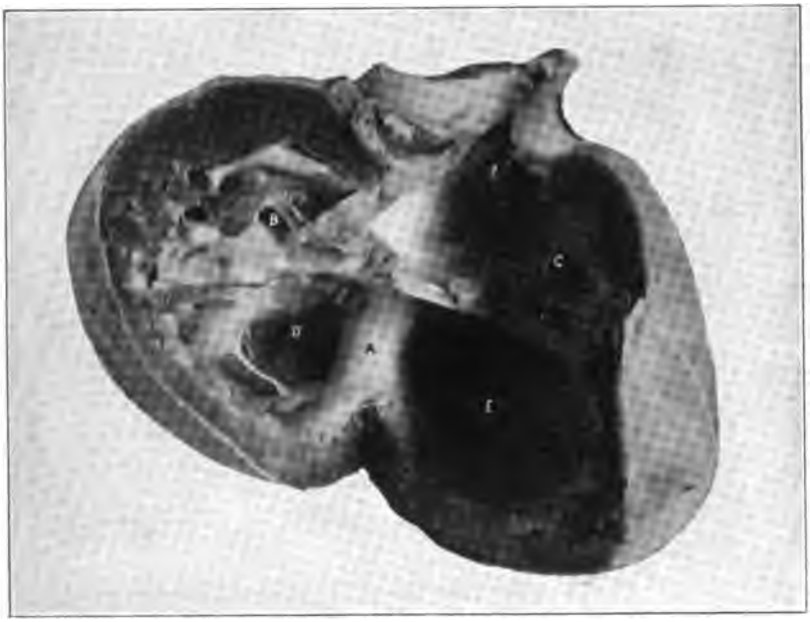


Fig. 6. Cor triloculare biatriatum. *a*, septum trunci; *b*, left auricle; *c*, right auricle; *d*, left ventricular portion of common ventricle; *e*, right ventricular portion of common ventricle.

ventricular orifice being 3 cm. in circumference. A rough estimate of the capacities of the two auricles would make that of the right three times that of the left. The right valve presents three flaps and several thickenings varying from 1 to 6 mm. in size. The left valve presents two main flaps which are broken up into a number of smaller ones by the insertion of anomalous chordæ tendineæ. The interauricular septum forms a common site of origin for both valves and a common papillary muscle receives chordæ from each of these valves.

There is but a single ventricle without any evidence of attempt at division in two by an interventricular septum. The walls are thick and heavy and present large papillary muscles and columnæ carneæ. The auriculo-ventricular orifices present at the posterior part of the ventricle, the orifices of the great vessels at the anterior part of the ventricle, the aortic on the right, the pulmonary on the left. These orifices are separated by a thick, heavy band of muscle fibers passing anteriorly and posteriorly between them and represent the proximal septum trunci.

I am much indebted to the anatomical department of the College of Physicians and Surgeons for permission to reproduce the reconstructions indicating the divisions of the truncus into the aorta and the pulmonary artery.

ADDITIONAL CASE REPORT

Since the report of the preceding case of complete transposition of the arterial trunks, it has been our good fortune to find another instance of the same pathological condition. The two hearts are almost identical and present pictures that agree in every essential point. In the second instance the child lived for about six hours after birth. There was a great deal of cyanosis affecting particularly the extremities, the hands and feet being a deep indigo.

Upon opening the thorax the organs were found to be intensely engorged and congested, the lungs being almost black. As soon as the pericardium was opened, the transposition was recognized and the organs were removed *in toto*. As in the previous case the aorta arises from the right ventricle, passes upward, giving off the great vessels from its convexity and receiving on its concavity the patent ductus arteriosus as it passes over the pulmonary artery. The pulmonary artery shows the same arrangement as we have seen previously, dividing into its two pulmonary arteries under cover of the aortic arch, the left one in turn giving off immediately the ductus arteriosus.

Upon opening the right auricle, it is found to be large and dilated; the foramen ovale is patent along its anterior edge. The left auricle is considerably smaller than the right. The right ventricle presents the origin of the aorta. Passing posteriorly and slightly to the right behind the origin of the aorta is a thick heavy band of muscle fibers which represents the lower margin of the primitive septum trunci. It serves to separate the aorta and the right auricle and from its posterior aspect it gives origin to one of the flaps of the tricuspid valve. The origin of the two coronary arteries can be seen in the posterior and left sinuses of Valsalva of the aorta. The flaps of the mitral and tricuspid valves are normal in structure and number. The left ventricle presents the origin of the pulmonary artery arising anteriorly and close to the interventricular septum. The interventricular septum presents no defects.

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Discussion:

DR. MACCALLUM: I think Dr. Riley has covered the ground pretty thoroughly. There is one striking point in this connection and that is the mathematical regularity with which certain principles are carried out in transpositions of the great vessels. In making out his schema Rokitansky was able to prophesy anomalies of the great vessels and several types which had not been reported prior to this, but which he had figured as possible in his schema were later reported by different observers. A good deal of light can be thrown on this subject by cutting the hearts transversely close under the great vessels to show the disposition of the aortic and interventricular septa and by comparing them with pathological hearts cut in a similar fashion. A better idea of the relationships can be gathered in this fashion than by cutting the heart in the usual fashion through the valve flaps.

DR. LARKIN: I have had rather an interesting anomaly of the heart recently. The child had spina bifida. In these cases anomalies of the cardia are not infrequent. The child presented several abnormal conditions. This abnormality of the heart has never before been described. The charts of Dr. Riley give an idea of the disposition of the vessels. In my case the aorta leads directly into the right and also into the left ventricle, the pulmonary artery being normal. The aorta is normal until the base of the heart, where there is a lack of the septum. The aorta is bifurcating, one part goes to the right ventricle, one to the left.

DR. RILEY: The explanation is I think that the interventricular septum comes up from below and the septum trunci comes from above; they do not meet and the interventricular septum separates the aorta into two parts.

DR. LARKIN: There is no evidence of any upper septum at all.

DR. RILEY: You have a division of the truncus into two vessels.

DR. LARKIN: The pulmonary goes into the right ventricle, the aorta into the left. There is no abnormality of the pulmonary.

FIVE CASES OF PRIMARY CARCINOMA OF THE APPENDIX

A. B. EISENBREY, M.D.

(From the Pathological Department of St. Luke's Hospital, F. C. Wood, Director)

The five cases of primary carcinoma of the appendix to be reported were found among approximately eighteen hundred appendices examined in the pathological laboratory of St. Luke's Hospital during the past three years. The incidence of this lesion among our cases is thus seen to be 0.3 per cent. as contrasted with the 0.4 per cent. and 0.5 per cent. reported by other observers. Four of the five cases were females and the ages of the patients varied from nineteen to sixty years. In one of the cases the lesion was found at autopsy, and in but one of the remaining four cases was attention directed to the appendix before operation. In two instances only was the presence of carcinoma suspected from the gross appearance. In two of the appendices the lesion was situated in definite thickened areas at about the mid-point in the organ; in two others it was found in the enlarged clubbed tip, and in the fifth in the constricted fibrosed tip. In none of the cases was there macroscopical evidence of extension through the serous coat of the organ, nor was the organ adherent to the surrounding structures; no other carcinomatous lesion was found elsewhere in the body, either at operation or at autopsy. The appendices ranged from 4.5 cm. to 6 cm. in length and from 0.5 cm. to 1 cm. in diameter, except at the site of the lesions, of which the largest was 1.2 cm. in diameter.

Microscopically the lesions all showed the most frequently described type of appendiceal carcinoma: destruction and replacement of glandular and lymphoid structures by a rather dense fibrous stroma, supporting islands and strands of cells of the spheroidal epithelial type which also invaded the muscularis and in several of the cases were found in the subserous structures. In two of the cases the cells were smaller, fewer large islands were found and the growth in general was of a more scirrhus type.

(We had a sixth case of carcinoma of the appendix which is not included in this report. It was of the gelatinous type and while the chief growth and origin appeared to be in the appendix the cæcum was also extensively involved so that it is not justifiable to class it as primary in the appendix.)

CASE I. (Path. No. 8084.) Female, aged twenty-five, gave a history of pelvic disturbances following birth of child six and a half years before but showed no symptoms referable to the appendix. At operation for the correction of retroversion of the uterus the appendix was found to be constricted at the distal fifth of its length and was removed. The carcinomatous growth was seen, in longitudinal section of the constricted portion, to involve the area nearest the base where its relation to the mucosa was well shown. The region of the tip was free from the growth. The islands of cells in this case were large with rather large and vesicular cells.

CASE II. (Path. No. 8415.) Male, aged sixty, was operated upon for acute appendicitis of three days duration. He gave no history of any previous disturbances of like nature. At operation the appendix gave evidences of a chronic inflammatory process with an added acute congestion. The middle portion of the organ was slightly thickened and densely fibrosed. The cells were of a smaller type, the islands small but numerous, with a well marked invasion of muscularis and subserosa.

CASE III. (Path. No. 10845.) Female, aged thirty-one, gave a history of symptoms referable to the pelvic organs, extending over a period of five years. No symptoms were directly referable to the appendix. At operation for double salpingitis and retroverted uterus the appendix was found thickened throughout, the serosa was congested but smooth, and there was considerable clubbing of the tip. In this case the cells were of the large clear type, the cell nests large, and the invasion of the subserosa most marked.

CASE IV. (Path. No. 11554.) Female, aged forty-two, was operated upon for fibromyomata of the uterus and the appendix removed incidentally. There was marked clubbing of the tip and considerable congestion and a slight exudate on the serosa over it. The lesion was well confined to the region of the mucosa and submucosa. The cells and cell-groups were of the large type.

CASE V. (Autopsy No. 1153.) Female, aged nineteen, died of septicæmia following a septic endocarditis of probable rheumatic origin. The appendix appeared normal externally except for a hard thickening about 1 cm. in diameter at its middle. On section this was of dense fibrous structure with a rather granular surface of yellowish color, an appearance noted in the other cases of this series which we believe characteristic of this type of lesion. In this case the growth was of the scirrhus type, the cells were small and the invasion to the serosa well marked.

Discussion:

DR. EWING: In connection with reports of cases of carcinoma of the appendix it has been objected that while these processes have the morphology of carcinoma they are not genuine malignant tumors, but merely atypical overgrowth of aberrant epithelial rests or of displaced portions of mucosa which possess a very limited power of proliferation. Many of the cases fail to show the clinical features of a malignant tumor and it may well be that some of the tumors are practically at a standstill when observed. I think, however, that they are properly regarded as true carcinomas of their own peculiar sort. We have no right to demand that they should show all the characteristics of malignant tumors in other parts of the body. They most resemble the basal cell carcinomas.

DR. EISENBREY: There are mitotic figures in these specimens, but they are hard to find. In none of the cases were there enlarged glands of the meso-appendix, nor was there any demonstrable carcinoma elsewhere, except in the case of gelatinous carcinoma, where the cæcum was also involved. That case is not included in this series.

DR. PAPPENHEIMER: Are there any cases in the literature in which widespread metastases have been reported?

DR. EISENBREY: Yes, there are some cases where there is active recurrence. So far as this particular type goes, I do not believe there are any recurrences.

EXTRA-MEDULLARY MYELOID CHANGES IN ORGANS

W. G. MACCALLUM, M.D.

Dr. MacCallum presented preparations from two cases to illustrate the appearance of myeloid tissue in organs other than the bone marrow. One of these was a case of so-called osteosclerotic anæmia in which the destruction of practically all of the bone marrow by metastases from a carcinoma of the prostate resulted in an extreme anæmia and the spleen and liver were found laden with myeloid cells. The venules of the spleen were especially filled with myelocytes. The second case was one of myeloid leukæmia in which distinct nodules of myeloid tissue including megalocaryocytes were found in the liver. Two views have been held as to the origin of these cells; one being that elements originally present in most tissues and capable of production of cells of the blood are reformed by a sort of reversion to embryonic conditions; the other being that myeloid cells are swept out of the bone marrow and in case of necessity colonize in other organs and there produce the elements of the blood. In the case demonstrated the latter view seems most probable.

Discussion:

Dr. Pappenheimer: I would like to ask whether, in the case of extreme sclerotic anemia, any myeloid cells found their way into the circulation.

Dr. MacCallum: There were foci of the same sort. I don't believe any went into the circulating blood. There were some in the adrenals.

Dr. Field: These myeloid changes are very frequently found in areas of arterio-sclerosis in the aorta, in cases which have shown only slight anemia. It would seem to me that Maximow's *Wanderzellen* can give rise to both series of blood cells when the nutritive and pressure conditions are favorable, in the same way as they do in embryonal life.

Dr. Ewing: Does Dr. MacCallum know of any definite instance in pathology where an embryonal function or tissue, once passed through its normal course of development to the adult, experiences a reversion to the embryonal condition?

Dr. MacCallum was glad that Dr. Ewing expressed himself as opposed to the idea of a reversion of cells to embryonic form and function, since he had seen no evidence of it. The explanation is difficult in the case of bone marrow formed in necrotic tissue found in the walls of blood vessels and the lung, but in other cases the theory of colonization seems better supported.

LATE RESULTS IN ACTIVE IMMUNIZATION WITH DIPHTHERIA TOXIN-ANTITOXIN AND WITH TOXIN-ANTITOXIN COMBINED WITH DIPHTHERIA BACILLI

WM. H. PARK, M.D., AND ABRAHAM ZINGHER, M.D.

(From the Research Laboratory and the Willard Parker Hospital, Department of Health, New York City)

The property of diphtheria toxin-antitoxin mixtures to produce immunity in animals has been known for a number of years, but its application to human beings was not practised until Von Behring recommended it in May, 1913. The early reports of Von Behring and his co-workers were so favorable that we determined to make a similar attempt at the Willard Parker Hospital.

Several series of patients were injected with mixtures of diphtheria toxin and antitoxin, in such proportion that the mixture was either overneutralized, just neutral or slightly toxic to the guinea-pig. In the last series of cases (Group III) we used a mixture consisting of 85 per cent. of the L_+ dose of toxin to each unit of antitoxin, to which was added a vaccine made from killed diphtheria bacilli.

In a previous communication we published the following conclusions, derived from the results obtained in the immunization of two groups of scarlet fever patients who were injected with toxin-antitoxin:

1. Those who had a natural antitoxic immunity produced a decided increase in the amount of antitoxin in a short time (seven days), even after a single injection of toxin-antitoxin.

2. Those who had no natural antitoxic immunity were quite resistant to active immunization. Only 25–30 per cent. showed decided antitoxin production within four to six weeks after injections of toxin-antitoxin. A larger proportion produced a trace of antitoxin, and gave a fainter Schick reaction than in the original test. The reaction was still positive, however, and they were not considered immune.

3. We concluded, therefore, that toxin-antitoxin could be used

where no immediate danger of infection was present. Non-immune individuals exposed to diphtheria, however, should be protected with antitoxin.

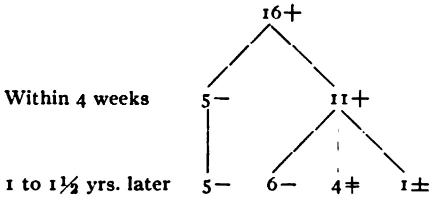
Observations on our chronic tube cases, who developed slowly, but almost uniformly, an antitoxic immunity, led us to retest with the Schick reaction those who were at first partially or totally refractory to immunization with toxin-antitoxin.

From Group II (toxin-antitoxin) we retested sixteen cases, from Group III (toxin-antitoxin plus bacterial vaccines) fifty cases, at intervals of from two months to one and one-half years after the immunizing injections.

In Group II, a series of ninety patients was originally injected who had no natural immunity, as shown by blood examination for antitoxin and by a positive Schick reaction. Of the ninety, twenty responded to the injection of toxin-antitoxin with the production of sufficient antitoxin to protect against diphtheria; seventy failed to respond to this degree, though some gave a fainter Schick reaction than they had shown before the immunization. Among the twenty no cases of diphtheria developed; of the seventy who produced very little or no antitoxin, seventeen developed clinical diphtheria twelve days and more after the first injection.

From Group II, sixteen cases were subsequently retested one to one and one-half years after the injections of toxin-antitoxin.

EARLY AND LATE SCHICK TESTS IN GROUP II.



— indicates negative Schick reaction.
∓ indicates faint Schick reaction.
± indicates moderate Schick reaction.
+ indicates fully developed Schick reaction.

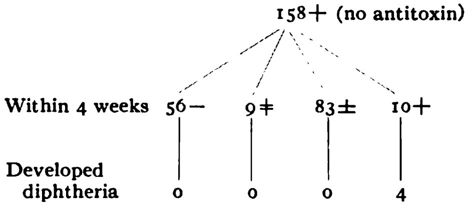
Five of the sixteen who had shown an early and decided production of antitoxin, again gave a negative Schick reaction. Of the eleven who had failed to respond at first to the immunizing injections, six now gave a negative, four a very faint and only one a positive Schick reaction.

Group III consisted of one hundred and fifty-eight patients, who had originally given a positive Schick reaction. These patients were immunized with a mixture of toxin-antitoxin and a vaccine made of killed diphtheria bacilli. Three to four subcutaneous injections were given at intervals of three to four days, each injection consisting of 1 c.c. toxin-antitoxin (85 per cent. L_+ dose of toxin to each unit of antitoxin), and 1,000,000,000 bacteria. One c.c. of the mixture injected subcutaneously into a guinea-pig produced a slight local induration and paralysis in ten days.

EARLY RESULTS IN GROUP III

158 cases

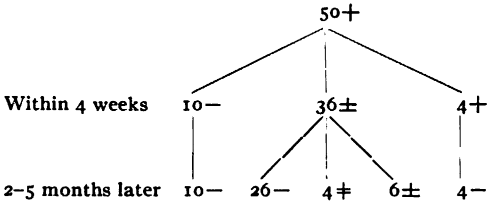
No antitoxin (+ Schick) before immunization



Of the one hundred and fifty-eight injected patients, fifty-six or 35.5 per cent. produced enough antitoxin within four weeks to give a negative, and nine, or 6 per cent., a very faint Schick reaction. Of those who were partly or totally refractory, eighty-three, or 52 per cent., gave a moderately positive, and ten, or 6.5 per cent., a strongly positive Schick reaction. Of the ten, four developed clinical diphtheria.

To note any late development of immunity, we retested from two to five months after the injections, fifty cases of this group.

EARLY AND LATE SCHICK TESTS IN GROUP III.

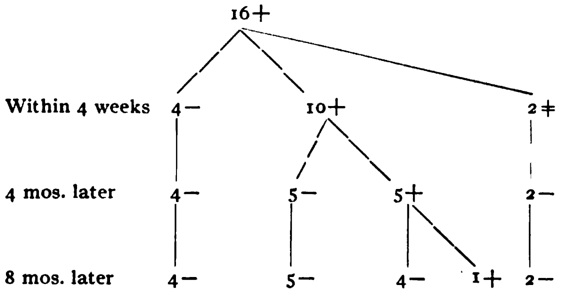


Ten of the patients who had given a definite early production of antitoxin continued to show a negative Schick reaction. Of the thirty-six patients who had produced only a trace of antitoxin, not sufficient to inhibit the Schick reaction, twenty-six now gave a negative, four a very faint and six a moderately positive reaction. The remaining four who were refractory at first, now gave a negative Schick reaction.

Grouping together the early and late successful results in this series of cases, we find that forty out of fifty susceptible individuals had developed an antitoxic immunity after the injections of toxin-antitoxin plus vaccine. The remaining ten gave a reaction which was moderately positive in six and faintly positive in four cases. These cases probably had developed some bactericidal immunity from the bacilli injected.

In addition to these, we also immunized with toxin-antitoxin, a series of sixteen normal children (Group IV), who had given a positive Schick reaction. These children were injected about a year ago, and retested with the Schick reaction four and eight months after the immunization.

EARLY AND LATE SCHICK TESTS IN GROUP IV (NORMAL CHILDREN)



Within four weeks after the injections, only four, or 25 per cent., gave a negative Schick test. Four and eight months later, these four still gave a negative reaction, thus indicating a probable lasting immunity. Ten children, who were refractory at first, now showed the following interesting results: at the end of four months, five gave a negative and five a positive Schick reaction. At the end of eight months, nine showed a negative reaction and only one was still positive. Two of the sixteen children who had given a partial early response, were found immune at the end of four and eight months.

As control, we had a group of six children who had received antitoxin only a year ago. At the end of eight months, five of the children still gave a positive Schick reaction, and one only had now become negative.

Another group that may serve as control consisted of sixty children who had received vaccines made only from diphtheria bacilli. Retested from five to eight months after the injections, forty gave a positive and twenty a negative Schick reaction.

Our results in active immunization up to the present time have led us to the conclusion that, while the toxin-antitoxin injections cannot be depended on to prevent diphtheria in the presence of immediate danger of infection because of the slow development of immunity in a majority of the cases, yet the injection is of value in rendering a community or school population immune to the danger of infection from future exposure up to a period of possibly several years.

CONCLUSIONS

1. Individuals who before treatment give a negative Schick reaction are immune probably for life, and therefore it is not necessary to inject them either with antitoxin or toxin-antitoxin.

2. Those who give a positive Schick reaction and are exposed to diphtheria and in immediate danger, should receive either antitoxin alone, or if a longer protection is desired, both antitoxin and toxin-antitoxin.

3. For general prophylaxis against diphtheria excluding im-

mediate contacts, a mixture of toxin-antitoxin alone (85–90 per cent. of the L_+ dose of toxin to each unit of antitoxin) or toxin-antitoxin plus vaccine of killed diphtheria bacilli, is recommended. The dose is 1 c.c. of toxin-antitoxin and 1,000,000,000 bacteria injected subcutaneously and repeated three times at intervals of six or seven days. Sufficient time has not yet elapsed to judge the value of adding the injections of the bacilli to the toxin-antitoxin.

4. The early and the late results of active immunization should be determined with the Schick test. Early results are those obtained by the application of the test within four weeks, late results from four months to two years after the immunizing injections.

Discussion:

Dr. Field: Dr. Zingher's paper is extremely interesting in that it gives us an explanation from the laboratory as to why diphtheria is a disease of the younger years and is comparatively rare among adults. When we realize that 5 per cent. of all school children harbor the diphtheria bacillus in their throats, it is readily understood why such a large proportion should be found to be immune by the Schick test.

Dr. Zinsser: The antitoxin contents of the blood are relatively high during the first year of life, then lower, again increased toward adult life, falling again in old age.

Dr. Field: It falls in with the Schick test.

Dr. Zinsser: An interesting point in Drs. Park and Zingher's paper is that they are using mixtures which are not over-neutralized, namely, an L_+ dose with one unit. In Behring's experiments I believe the mixtures were considerably over-neutralized.

Dr. Field: In regard to the production of antibodies by mixtures of toxin and antitoxin, I would like to call the attention of the Society to the fact that Dr. Park reported on this point before the Society fourteen years ago. The work had been done two or three years previous to his making the report, and when Ehrlich visited the Research Laboratories he was surprised to discover this point; I well remember his animated conversation with Dr. Park in regard to it.

Dr. Zingher: Antitoxin production is abundant in the horse after injections of neutralized or over-neutralized mixtures of diphtheria toxin and antitoxin. Similar results are obtained in human beings who are naturally immune. There is probably a dissociation of the toxin-antitoxin mixture *in vivo*, and a ready response of the tissue cells to the antigen. It is important to have the right proportion of toxin and antitoxin in the mixtures used for active immunization. A mixture that is only slightly toxic to the guinea pig gives the most satisfactory results in non-immunes. Von Behring did not give in detail the exact proportions of toxin and antitoxin. He injected neutral and slightly toxic mixtures. The doses he recommended were only a fraction of the ones we used.

Dr. Zinsser: We are learning all the time that we can immunize with antigen-antibody combinations in which apparently no free antigen is present. This is, of course, the principle underlying immunization with sensitized vaccines as introduced by Besredka and the early experiments of Kraus and others on protection with sensitized cholera spirilla. It seems hard to understand this unless we assume dissociation of the antigen-antibody complex within the body.

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FOUR CASES OF PRIMARY CARCINOMA OF THE LUNG

ROBERT A. LAMBERT, M.D.

(From the Pathological Laboratory of Montefiore Hospital, New York City)

The relative infrequency of primary carcinoma of the lung is the reason for bringing before the Society four specimens which were encountered among less than fifty autopsies performed at Montefiore Hospital during the past eight months. Such a high incidence is very unusual and is undoubtedly accidental, in view of the reports of incidence of 0.2 to 1.0 per cent. from many other institutions. A review of Dr. Adler's excellent monograph on "Primary Malignant Growths of the Lungs and Bronchi," in which all of the published cases have been collected, has convinced me that our four cases belong to well recognized types of lung carcinoma. The first two are distinct squamous celled epitheliomata, prickle cells and keratinizing "pearls" being frequently encountered. The third belongs to the group of adenocarcinomata arising in bronchial glands. The cells exhibit in many places an active secretion of mucus. The fourth is a papillary adenocarcinoma, the cells of which possess in places definite cilia.

An interesting feature of these cases is the difference in the tendency toward metastasis formation. The first two tumors showed no metastases. The third, on the other hand, besides an extensive local spreading, metastasized to the axillary, cervical and abdominal lymph nodes, and liver; while in the fourth case, a papillary ciliated epithelial tumor, metastatic nodules were present, not only in most of the abdominal viscera, but also in various bones (vertebræ and ribs especially) and in the brain.

The squamous cells of the first two tumors, which are generally thought to arise from the cylindrical epithelium of the bronchi, must be regarded as having strayed far from the parent type; that is, the cells are, in this sense, very atypical. Yet, in their behaviour they appeared less malignant than the more nearly typical cells of the third and fourth cases. Indeed, it was the ciliated epithelial cells of the last case that manifested the most pronounced tendency to metastasize.

Complete reports of these cases will be published elsewhere.

Discussion:

Dr. Norris: I was greatly interested in Dr. Lambert's presentation. He has been very fortunate in finding four cases of primary carcinoma of the lung in such a small series of autopsies. At Bellevue we have records of approximately 4,800 autopsies, and I have seen but two cases of supposed primary tumor of the lung. One of these cases occurred in October, 1904. Recently in looking over the records of primary carcinomas of the lungs, the protocol of the case does not mention the condition of the œsophagus. The tumor was a frank epithelioma. I question very much that the case represents a true primary epithelioma or carcinoma of the lung. The second case occurred more recently. There was a large tumor mass at the hylus of the lung, involving the bronchial and tracheal lymph nodes; there were no metastases with the exception of a small nodule in the small intestines. The microscopic picture in both cases was similar, namely, the tumor was acinous in structure with production of mucus. I do not feel sure that the small nodule in the intestine was not the primary seat of the growth. This autopsy was done very carefully and it was thoroughly sifted.

Dr. Schultze: I held an autopsy on one of Dr. Adler's cases where there was a terminal hæmorrhage. I took issue with the doctor as to its being an endothelioma. I considered the case one of carcinoma of the lung. Since then I have seen two cases which may be primary endothelioma. One started in the bronchial lymph nodes with metastases into the bone marrow. Probably both types of tumors exist.

Dr. Lambert: I think Dr. Norris's criticism is pertinent. It is possible that one of these cases may not be primary in the lung. There was a small erosion of the œsophagus. But I do not think the tumor could have been an œsophageal growth. It would be remarkable for a tumor of the œsophagus to infiltrate the lung as extensively as this one did. I do not think there is any doubt about the other three tumors being primary in the lung. The autopsy was complete in each case and a careful search was made for other possible sites of origin.

REPORT OF AN UNUSUAL CASE, PRESENTING PRIMARY, MULTIPLE, BENIGN, MEDULLARY, GIANT-CELL BONE TUMORS

H. S. MARTLAND, M.D.

(From the Pathological Laboratory, Newark City Hospital)

Introduction

Sarcomata occurring in or near the ends of long bones, endosteal, medullary or myeloid in character, and showing a microscopical picture characterized by numerous giant-cells, having abundant, dark staining and often vacuolated cytoplasm, numerous and regular nuclei with absence of mitotic figures, and a stroma consisting of fibroblastic granulation tissue without active mitoses, dilated and engorged blood-vessels, hemorrhage in perivascular tissue, erosion and disintegration of bone trabeculae, have been recognized for many years under such terms as medullary or myelogenous giant-cell sarcomata.

Adami and Bland Sutton have objected to their classification as sarcomata on the grounds that the type of giant-cell encountered in this tumor is a cell resembling the osteoclast or myeloplax occurring in normal bone marrow, and also, from the well known clinical fact, that these tumors were much less malignant than periosteal sarcomata, and in many cases distinctly benign.

Bloodgood, in 1902, was the first in this country to seriously study this tumor, and he concluded that he did not believe that this type of tumor ever caused metastases, but would recur *in loco* when not thoroughly removed, a fact that was recognized as early as 1895, by Koenig, Bergmann and Mikulicz. Bloodgood therefore concluded that it was not a sarcoma, and for a better name. termed it medullary giant-cell tumor.

Mallory considers the giant-cells encountered in this tumor as foreign-body giant-cells, formed by the fusion of endothelial leucocytes, and believes that in forming a diagnosis, they should be considered as entirely of secondary value. Mallory would attempt to trace the tumor back to its type cell, say the fibroblast, and the tumor's malignancy would be determined by the presence or absence of active mitosis.

Barrie, in 1913, called attention to the characteristic macroscopic and microscopic appearances of these tumors, which are entirely different from that of malignant periosteal tumors. Barrie believes that the process is not a tumor formation at all, but merely a chronic non-suppurating form of osteomyelitis, therefore he has named the lesion chronic non-suppurative hemorrhagic osteomyelitis.

The table below gives a condensed idea of the clinical and pathological facts known, in so-called medullary giant-cell sarcoma or chronic hemorrhagic osteomyelitis of Barrie.

CLINICAL FACTS.

Situation: Ends of long bones, especially upper end of tibia.
Trauma: Definite history in large majority of cases.
Age: Young people, large majority.
Onset: Usually sudden.
Malignancy: Will recur *in loco* after incomplete removal, but will never form metastases.

Roentgenograms: Expansive, abrupt and circumscribed tumors; distention may be great, bone shell preserved.

PATHOLOGICAL FACTS.

Gross Appearance: Tumors usually confined within periosteum. Definitely circumscribed, not infiltrating. Easily removed from bone shell. Are distinctly vascular, resembling young granulation tissue, friable, soft and ooze. Resemble currant-jelly or fresh cut liver. Later this picture may change, and the tumor become cystic.

Microscopic Appearance: Giant-cells of foreign-body or osteoclastic type.

Nuclei: Regular in size and shape. Numerous. Absence of mitotic figures.

Cytoplasm: Dark staining. Abundant and often vacuolated.

Stroma of Tumor: Suggests young granulation tissue, with endothelial-lined blood-spaces separated by connective tissue stroma, filled with spindle and round cells, showing few if any mitotic figures.

I wish to present the histories, clinical courses, and pathological findings in two cases presenting bone tumors of the above described type. Both patients are from the surgical service of Dr. F. R. Haussling at the Newark City Hospital.

The first case presents an expansive tumor involving the lower end of the right tibia, of over eight years' duration, which on microscopical examination shows the typical picture of the so-called medullary giant-cell sarcoma. I present this case to demonstrate the benign character of this well-known tumor.

The second case presents multiple expansive bone tumors, the clinical picture resembling that of multiple myeloma; the microscopical appearance of the tumors, however, is that of the so-called medullary giant-cell sarcoma. Multiple tumors presenting this microscopical appearance have hitherto been undescribed.

CASE I. *History:* A. L. Male, Italian; age twenty-eight years; barber. Past history: Had chancre when eighteen years old. In 1907, he twisted his right ankle while playing ball; leg was put in plaster three weeks; he then received luetic treatment for one month; plaster cast again applied for two months; lower end of tibia curetted, and after wound healed he was discharged as cured. In 1908, the swelling and pain returned in the ankle; the tibia was again curetted, a diagnosis of osteomyelitis made and he was discharged with a persistent sinus. In 1910, he fell and again injured ankle; he was at this time admitted to the City Hospital. Subsequent history: The lower end of the tibia was curetted, and microscopical examination showed myelogenous giant-cell sarcoma. The wound healed, but old sinus persisted. During 1911, the tibia was curetted four times for recurrence. In 1915, the tibia was again curetted for recurrence in and about sinus, and the surgeon noted that the original bone cavity was no longer filled with red granulation tissue, but consisted of several cystic cavities filled with clear fluid. Between these last curettages, he has been able to work as a barber, and his general physical condition is excellent.

Summary: The points of interest in this case are:

(1) A single, expansive tumor of the lower end of the tibia.
(2) Slow growth, over eight years' duration.
(3) Eight curettages for recurrence, which is always *in loco*.
(4) Absence of metastases.
(5) Excellent physical condition of patient.
(6) Typical gross appearance of tumor is changing from that of a cavity filled with red granulation tissue (chronic hemorrhagic osteomyelitis of Barrie) to that of bone cysts filled with clear fluid (chronic fibrocystic osteomyelitis of Barrie).
(7) Typical microscopical appearance of the so-called medullary giant-cell sarcoma or chronic hemorrhagic osteomyelitis of Barrie.

CASE II. *History:* T. K. Female; age twenty-five years; married; housewife. Family history: Negative. Past history:

Had measles in childhood; venereal diseases denied; has had four normal births, one miscarriage. In February, 1914, patient had a miscarriage; she lost considerable blood and has never been well since. She complained of weakness, dyspnœa, and palpitation on exertion. In August, 1914, she noticed a lump growing in the inner angle of right eye; this was the first tumor mass noticed. In October, 1914, she fell and broke right femur about six inches above the knee-joint. She was admitted to the City Hospital and the fracture united with good result. In February, 1915, she noticed a mass on the anterior surface of the left tibia, about its middle third, which grew for two months and attained the size of a hen's small egg; it then stopped growing. In May, 1915, she gave birth to a full term, healthy child. In June, 1915, she was admitted to the City Hospital, complaining of dyspnœa, weakness, cardiac palpitation and slight bone pains.

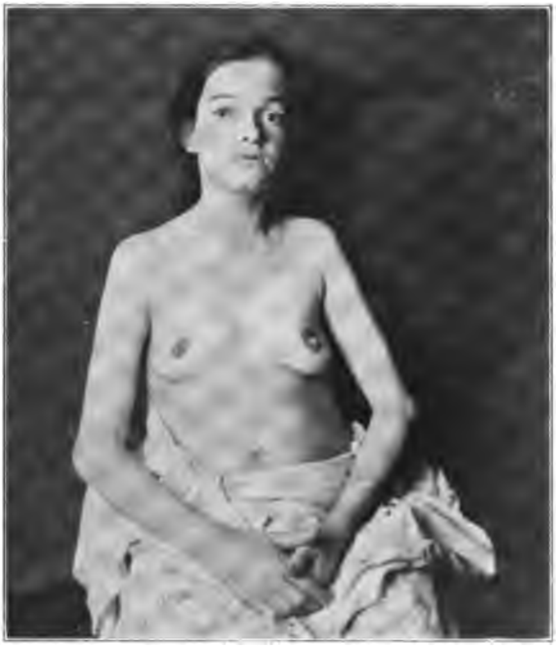

FIG. 1. Photograph of multiple bone tumor case, showing growth in right antrum.

Physical Examination: A well-developed female, fairly well nourished (Fig. 1). Nose, saddle shaped (due to trauma in childhood). Heart, apex in sixth interspace, outside nipple line; a loud mitral systolic murmur. Lungs, suspicious signs of early tuberculous involvement of left upper lobe. Rest of physical examination negative, except for the presence of palpable bone tumors. The right superior maxilla presented a hard tumor mass, which was infiltrating the inner wall and floor of the right orbital cavity. The left clavicle presented a hard mass the size of an English walnut at the sterno-clavicular joint. The right clavicle near its acromial end presented a hard tumor mass the size of an English walnut. The left tibia, on its anterior surface, and near its middle and upper third, presented a hard tumor the size of an English walnut.

Roentgenograms: Showed in addition to the palpable tumors, described above, expansive tumors in the following locations: In the seventh rib on the right side; in the right femur above the knee-joint (Fig. 2); in the right fibula on its upper third; and in the left fibula on its upper third (Fig. 3). Later roentgenograms showed a tumor developing in the right humerus just above the elbow (Fig. 4), and in the pelvis.

Laboratory Examinations: The following laboratory examinations were made:

Hæmoglobin	75 to 85 per cent.
Erythrocytes	4,020,000 (none abnormal)
Leucocytes	5,200 to 9,600 (none abnormal)

Blood Wassermann negative, three times
Spinal fluid Wassermann, negative once, with normal cell count and globulin

Urine: Several 24-hour specimens were examined, especially for Bence-Jones albumose, with negative results. The urine always showed a trace of albumin, with a small number of hyaline and granular casts. Otherwise the urine was normal.

Clinical Diagnosis: From the above clinical facts a diagnosis of multiple myeloma, cardiac dilatation, and failing compensation was made.

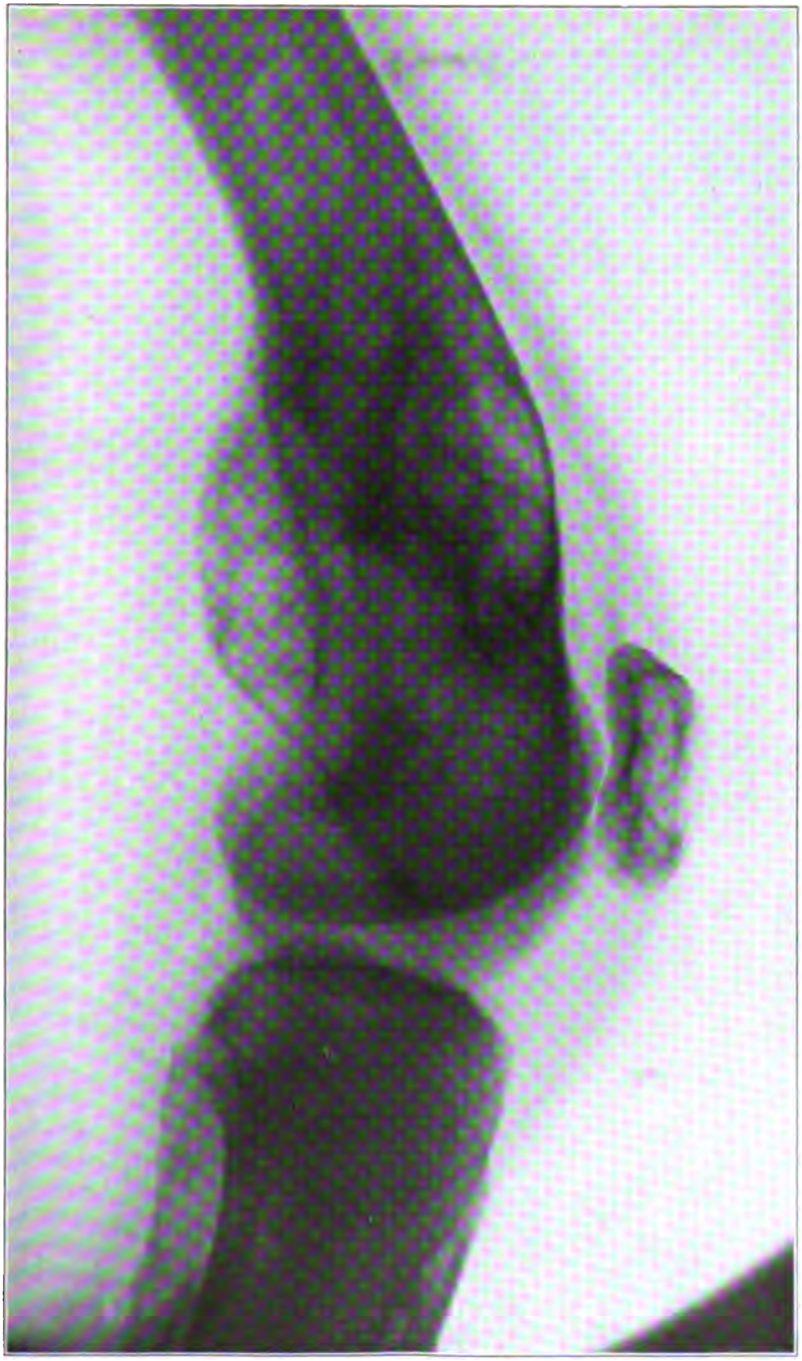

FIG. 2. Roentgenogram, showing expansive growth in lower end of right femur.

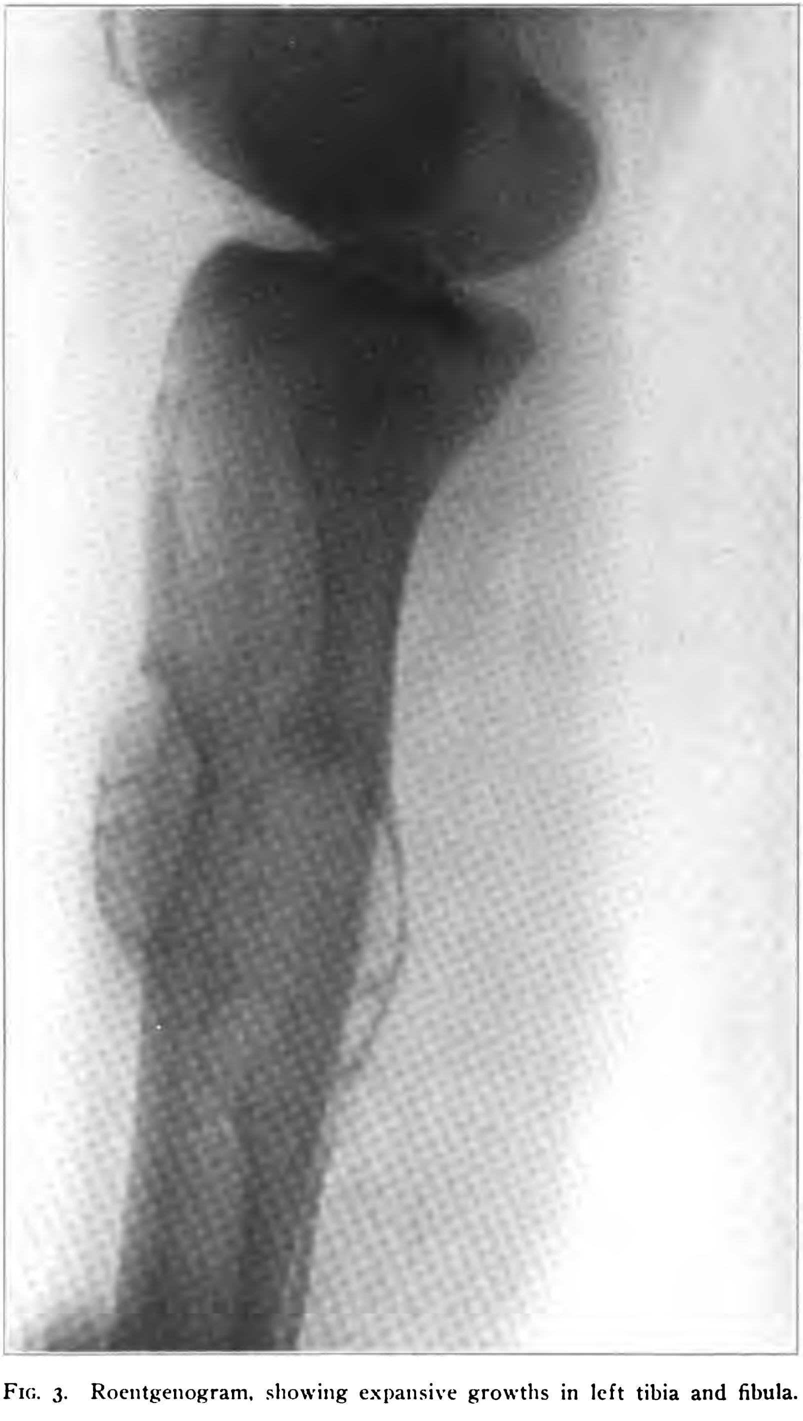

FIG. 3. Roentgenogram, showing expansive growths in left tibia and fibula.

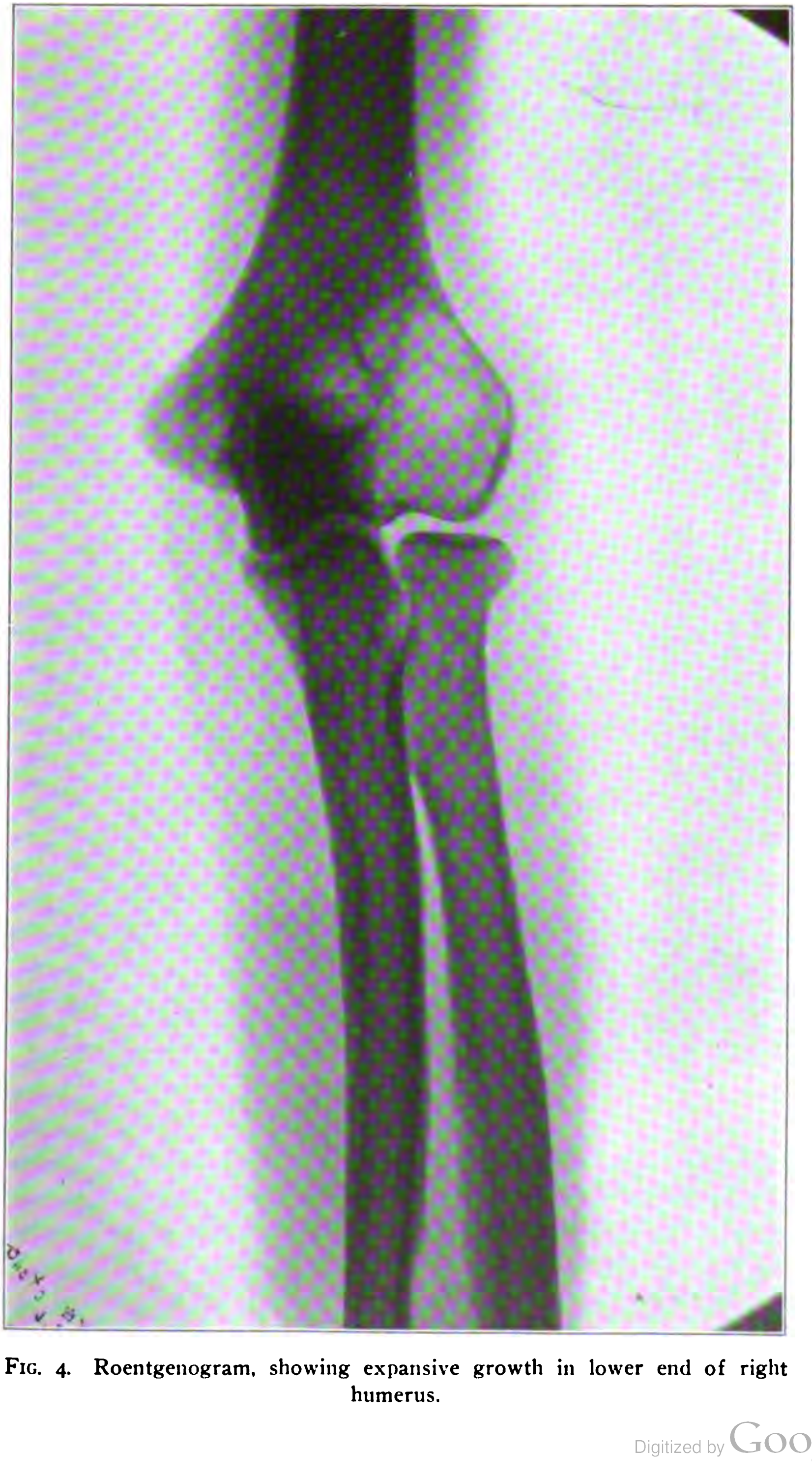

FIG. 4. Roentgenogram, showing expansive growth in lower end of right humerus.

Progress Notes: On June 28, 1915, in order to establish a positive diagnosis, Dr. Haussling curetted the growth on the left tibia. At the operation, he added the following notations to the history: "Incision revealed a small localized cavity, containing what appeared to be well-organized granulation tissue, reddish, and with considerable bleeding. Tissue curetted away, cavity swabbed with pure carbolic." Wound healed by first intention.

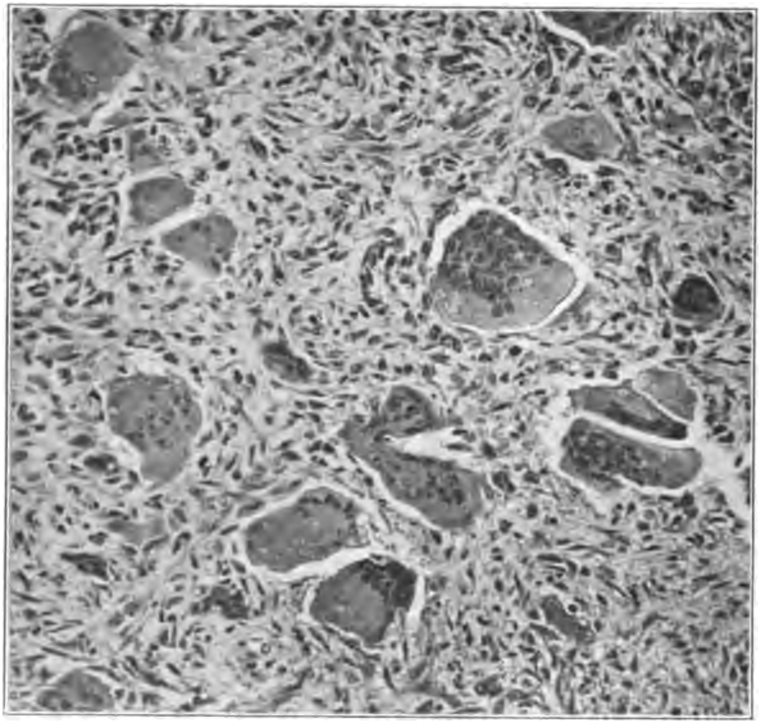

FIG. 5. Case II. One of the multiple tumors. Showing numerous giant-cells with fibroblastic stroma.

On microscopical examination of the curetted tissue, I was surprised to find the typical lesion of the so-called myelogenous giant-cell sarcoma. Believing this to be a benign lesion from our previous experience, Dr. Haussling decided to attempt to remove by the curette all the other tumor masses. On August 16, 1915, a second operation was performed and tumor masses in the following locations were exposed and curetted: right fibula; left fibula; left clavicle; seventh rib and right superior maxilla. The following notation was made by Dr. Haussling at the operation: "The lesion in the superior maxilla seems to involve the entire antrum and to be encroaching on the right nasal cavity. All tumors are well localized, the line of demarcation of diseased and healthy bone is quite sharp. The gross appearance is that of reddish, pale granulation tissue with considerable bleeding." All incisions healed by first intention. Microscopical examination of the various tumor tissue removed at the operation showed the typical picture of the so-called myelogenous giant-cell sarcoma (Figs. 5 and 6).

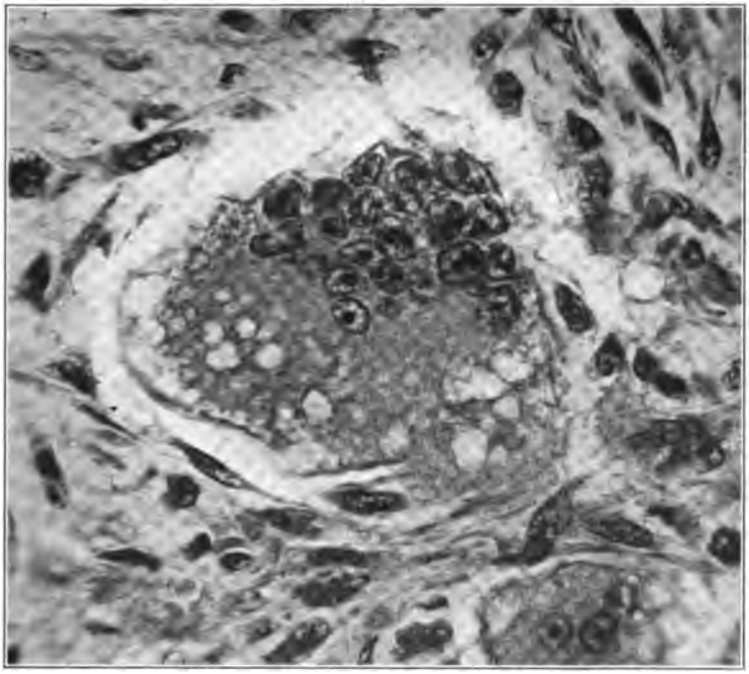

FIG. 6. Case II. High-power picture of typical giant-cell. Showing dark, abundant cytoplasm with numerous regular nuclei without mitotic figures.

Subsequent Notes: The patient's physical condition has greatly improved; her cardiac condition has become fully compensated. The tumors curetted still show in roentgenograms, and new ones are slowly making their appearance.

Summary: The points of interest in this case are:

(1) Multiple, primary, expansive, slow growing bone tumors.

(2) Tumors too numerous and situation too varied to infer trauma as an etiological factor.

(3) Tumors are well localized and circumscribed.

(4) There is absence of infiltration of soft parts by any of the tumors, in fact, none of them have yet broken through their bony shell.

(5) There has been very little bone pain.

(6) There is absence of visceral or other metastases.

(7) There is absence of emaciation, in fact the physical condition improves.

(8) All incisions over tumors healed by first intention, a condition not often seen in malignant bone tumors.

(9) All tumors had a typical or characteristic macroscopical appearance, namely showing solid and cystic portions, the former predominating, and resembling reddish granulation tissue with free bleeding.

(10) All tumors presented the typical microscopical picture of the so-called medullary giant-cell sarcoma or the chronic hemorrhagic osteomyelitis of Barrie.

Discussion:

DR. GEORGE BARRIE: In cases of hæmorrhagic non-suppurating osteomyelitis, or the so-called giant-cell myelogenous sarcoma that I have seen, the roentgenograms were different and did not resemble those shown by Dr. Martland from the multiple bone tumor case. As regards Mallory's views, I do not think the instances he has reported should be regarded as typical.

DR. COLEY: In any discussion pertaining to the question: whether myeloid tumors are benign or malignant, one must first determine what is meant by the term "myeloid sarcoma." One surgeon will call the small and sharply defined non-infiltrating tumors of the bones "malignant or round-celled sarcoma," while most of these are benign; and another includes in the term "myeloid sarcoma" all endosteal tumors, many of which are of extreme malignancy. One of the most important discussions of Tumors of the Long Bones was held at the Royal Society of Medicine in London (November, 1912). In the course of the discussion, Sir Frederick Eve stated that it was an error to consider all myeloid tumors as benign. Mr. Gilbert Scott, of London, stated that while the majority were benign, some of them were not only malignant, but showed a very marked degree of malignancy. Mr. R. C. Maybury cited two cases of myeloid sarcoma, observed at St. Thomas' Hospital, both of which were followed by general metastases which proved fatal.

The second case reported by Dr. Martland this evening had none of the clinical features of ordinary myeloid sarcoma of the long bones, nor did the *x*-ray picture point to such a condition. Dr. Coley stated that he believed the nearest approach to such a case as the one just observed was a case of Dr. Gibney's, in which slow-growing tumors developed in one long bone after another (usually followed by spontaneous fracture) until a large number of the bones were involved, finally causing death. The *x*-ray picture of these lesions is shown in Dr. Coley's recent paper on "Some Problems in the Early Diagnosis and Treatment of Sarcoma of the Long Bones" (*Annals of Surgery*, November, 1914).

Dr. Coley stated that he did not agree with Dr. Bloodgood and some others, who regard all cases of myeloid sarcoma of the long bones as benign. In his paper, referred to above, may be found a microscopical photograph of a tumor which occurred in the upper part of the humerus, in a boy ten years old. The growth attained its largest size three weeks after the injury. The clinical symptoms in this case pointed so clearly to a highly malignant sarcoma that Dr. Coley decided to perform immediately an amputation at the shoulder-joint, without waiting for a microscopical section. This he did, and later submitted a specimen of the tumor to Dr. Ewing, who pronounced it a giant-celled sarcoma of the epulis type showing only a moderate degree of malignancy. Dr. Ewing believed that Dr. Coley had made a mistake in amputating in a case that he thought could have been cured simply by curetting. In spite of the very radical operation, which was followed by a short course of toxin treatment, extensive metastases developed in the lung and the boy died within a year.[1]

Dr. Coley showed *x*-ray photographs of two cases of central sarcoma, recently observed at the Hospital for Ruptured and Crippled, one occurring in the humerus and the other in the tibia; both were classified by the pathologist as giant-celled sarcoma. Nevertheless, the clinical course of the disease proved it to be of a high degree of malignancy. In the first case there was extensive invasion of the knee-joint by a tumor of eight months' duration, and the whole lower end of the femur was destroyed. In the second case, the whole upper end of the femur was destroyed and extensive involvement of the fibula had taken place within seven months.

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[1] It is only fair to state that on later examination of the specimen by Dr. Ewing he inclined to the belief that the tumor belonged to the type of bone aneurism, of a very high degree of malignancy, and that his original diagnosis was undoubtedly erroneous.

Dr. Lambert: I was very much interested in the second case of Dr. Martland. I would like to ask if any other such cases have been reported. Regarding Dr. Coley's remarks, he tells us that he has seen examples of a typical giant-cell myeloid sarcoma of a highly malignant character. I wish he had brought sections from those tumors. I am inclined to think that he has confused the giant-cell myeloid sarcoma with the mixed sarcoma of bone, in which multinucleated cells are found. The latter tumor is, however, quite different from the former, both clinically and pathologically. The former is characterized not only by the presence of numerous large giant cells, but by an abundant connective tissue.

To regard the giant cells in myeloid sarcoma as accidental foreign-body giant cells, as Mallory does, appears to me to be without basis. Their formation is too uniform and constant to admit such an interpretation. Furthermore, foreign particles are not found in these cells.

Dr. Martland: In the multiple case the clinical appearance is like multiple myeloma. Histologically we find it resembling the single tumor case, therefore I was led to believe that these multiple growths were of the same nature and that they were benign in character. I do not believe that the tumors in this case will ever metastasize into the soft parts. The case will be carefully followed and will no doubt come to autopsy, and nothing will ever be satisfactorily determined until then. I think the antrum tumor will grow so large that it will invade the ethmoid cells and cause a meningitis, but I do not think she will ever die of metastases. I can find no evidence in literature of any case of multiple tumors of slow growth which have the same histological picture. As far as hypernephroma is concerned, we do not think it necessary to consider that in this case.

EQUILIBRIUM IN THE COMBINATION AND THE DISSOCIATION OF PRECIPITATES

RICHARD WEIL, M.D.

It has been shown by Linossier and Lemoine that if horse serum or other similar antigen be mixed with its specific precipitating anti-serum, the resulting precipitate never exhausts completely either of these two factors. Both of them can always be shown to be present in the supernatant fluid. This has been confirmed by Eisenberg and others. Moreover, the serum of immunized animals has been shown under certain circumstances to contain both precipitin and precipitinogen. By the original observers this was interpreted as an instance of the laws of mass action. Zinsser and Young interpreted it on the analogy of the protective action of a third colloid on two mutually precipitating colloids. Von Dungern attributed the observation to the presence of a multiplicity of antigens in the horse serum, or similar antigenic substance, with a corresponding mutiplicity of antibodies. The whole phenomenon, upon this basis, is reducible to a fallacy of technique. This theory was not supported by experiment and has not found further acceptance.

In order to test this theory a chemically pure antigen was prepared, namely crystalline egg albumen. When this is mixed with its anti-serum a precipitate results. The supernatant fluid can always be shown to contain either one of the two factors, egg albumen or antibody, but never both at the same time. This is true no matter how the proportions may be varied. With raw egg albumen and its anti-serum, on the other hand, the relations are the same as described by Eisenberg. The conclusion is drawn that Von Dungern's theory is correct. Under proper experimental conditions the precipitation reaction goes on to the complete exhaustion of either factor. "Equilibrium does not exist."

By means of absorption experiments it was shown that raw egg albumen contains not only the substance isolated as crystalline egg albumen, but also other antigens. Likewise, the antiserum to raw egg albumen was shown to contain a mutiplicity of antibodies. This confirms the previous conclusion.

In view of the fact that equilibrium in precipitation reactions does not exist, it is unnecessary to consider the explanations offered therefor. It is of interest to note, however, that the presence of either small or large amounts of serum, such as rabbit serum or guinea-pig serum, added to the above mixtures, does not in the slightest interfere with the completeness of the reaction. There appears, therefore, to be no analogy in this instance with the protective action of a third colloid.

Dissociation of precipitates has recently been studied by Chickering in connection with an extract of pneumococcus and its anti-serum. He used salt solution, or a one per cent. solution of sodium carbonate. He found that the extract contained no antigen, but did contain protective antibody and agglutinin. In my own experiments in dissociating the precipitate produced by horse serum, or egg albumen, and its appropriate anti-serum, different results were obtained. If the precipitate was treated with salt solution or sodium carbonate, antigen could always be demonstrated in the extract. It contained no precipitin, but did contain antibodies conferring passive sensitization. If the extraction was performed with trypsin or with rabbit's leucocytes, both antigen and precipitin could be demonstrated in the fluid. These reactions, like those previously described, have no analogy either in mass action or in colloidal chemistry.

Discussion:

Dr. Field: I would like to ask Dr. Weil how far digestion was carried with the leucocytes and trypsin. In Osborne and Mendel's work, when they carried digestion to completion there was loss of toxicity. This is used in testing the activity of proteolytic enzymes. In regard to the sodium carbonate and sodium chloride it might be due to precipitation.

Dr. Zinsser: In a matter of this kind time is needed for the discussion. I do not think it is logical, on the basis of these observations, to minimize the analogy between colloidal chemistry and serum reactions. In the precipitin reaction we have the influence of salts which is a striking analogy. We have the inhibition zones and the influence of quantitative relations on the reaction. We are dealing with obscure phenomena and must call analogy to our help and we have a right to reason from it, and the analogy to colloidal chemistry is too valuable to be disregarded. Five or six years ago Dr. Young and I were working on this problem. We did not consider the explanation of Von Dungern as likely. In our work we found that when we allowed the serum to stand it would precipitate spontaneously. The antibodies in the supernatant fluid would correspondingly diminish. This we thought was a reasonable argument that the antibody antigen complex came together by slow union. This, too, is analogous to colloidal suspensions where slow precipitation occurs even in the presence of a protective colloid. The mass action idea we did not consider correct because the sera themselves gave no complement fixation. This test is not absolute proof but it is the only indicator available. If the mass action idea was correct, then the serum itself should have contained antigen-antibody united as well as dissociated, and this complex, as far as we have any reason to argue from experience, should have fixed complement. I think that the precipitation phenomena of serum are too difficult at present to throw out peremptorily any help we get from colloidal analogy. So far as separation of the anaphylactic antibody from the precipitins goes, I may say that I have been one of those who for a number of years have maintained, both in experiment and in reasoning from the work of others, that there is only one antibody of which precipitation, agglutination, and other antibody reactions are different manifestations, depending upon the conditions under which the reactions are carried out and the nature of the antigen.

Dr. Field: In regard to the question of colloids and mass action, I used to use the term colloid chemistry, but I am sure we have got to be on our guard in making use of these names. I think the types of reactions between ions of extremely small size and molecules of larger size, and passing to molecules of organic compounds and then to colloidal gold and finally to clay and carbon, differ only slightly from each other.

Dr. Zinsser: I think that what Dr. Field says is true, and that no one has thought of disputing the perfectly self-evident statements he has made. That we are dealing with larger particles in one case and with smaller ones in the other, and, in the case of the larger particles, often with substances that are not chemically analyzable, is perfectly true, but it is also true that the methods of study of these two different kinds of substances are necessarily different, and since we believe that antigen-antibody reactions are of the latter nature, we apply these latter methods to their study.

Dr. Weil: The remark has just been made that the argument from colloidal reactions is really only a "cloud to cover our ignorance," and I think this remark truly conveys the gist of the whole matter. Instead of solving the problems of immunology we have been using the analogies of colloidal chemistry to veil our ignorance of the situation. I believe that the dangers and fallacies of such a course are evident from the results of the experiments which I have described this evening. The actual results of the immunological experiment do not in the least correspond to the analogy which had been drawn from the reactions of colloidal chemistry. This danger was long ago emphasized by Buxton, and even by Bordet, the arch-exponent of the colloidal interpretation. As regards the particular questions brought up, the problem of auto-precipitation would require too long a time for discussion here. I may say, however, that I do not agree with Dr. Zinsser's interpretation of this phenomenon. A full discussion of that phase will be presented in a publication of mine which will soon appear. There is another point in my paper to which Dr. Zinsser took exception, namely, the question of separation of the precipitin from the anaphylactic antibody. He feels that they are the same substance. I entirely agree upon that point. The two functions, however, namely, precipitation and passive sensitization, may be differentiated and separated. I have found that it is possible by heating precipitin to 72 degrees to deprive it completely of its precipitating effect, while the sensitizing value is retained almost unimpaired. In the terminology of Ehrlich the so-called ergophore group of the antibody, which is essential to precipitation, may be sacrificed, while leaving intact the so-called haptophore group, which alone is necessary for anaphylaxis. This observation, which is, I believe, of great importance for the understanding of anaphylaxis, has not been hitherto reported. It answers Dr. Zinsser's objection satisfactorily.

GROUPING OF MENINGOCOCCUS STRAINS BY MEANS OF COMPLEMENT FIXATION

MIRIAM P. OLMSTEAD

A study of literature on the meningococcus shows that while the complement fixation method has been used extensively in titrating serum and to a slight extent for diagnosis, except for work with the para-meningococcus practically no attempt has been made to use it in grouping strains of meningococci.

The work I am to report this evening has been done in collaboration with Dr. Phoebe DuBois, Dr. Josephine Neal and Miss Rose Schweitzer.

To obtain immune serum rabbits were inoculated by ten intravenous injections of live culture, administered every third day. The dose was increased from one fourth to a whole slant culture, washed off with normal saline. The cultures for inoculation were twenty-four hour growths in neutral veal agar.

Antigens were prepared according to the method suggested by Schwartz and McNeil for gonococcus antigen.

The total volume of the test was 0.5 c.c., one tenth that of the classical Wassermann. The anti-sheep system was used, cells in a five per cent. suspension, from one to two units of amboceptor, and guinea-pig serum for complement in a ten per cent. dilution. The system was carefully standardized each day by an amboceptor titration. Sensitized cells were used and the readings of antigen and antibody content titrations were made the next day, after the cells had settled.

The sera were freshly inactivated each day by heating for one half hour at 56° C.

Antigen and serum titrations were performed according to the technique described in Park and Williams' *Pathogenic Microorganisms,* 1914, pp. 184–187. The anticomplementary dose of antigen has been determined by testing decreasing amounts of antigen (0.3, 0.2, 0.1, 0.05 c.c.) in various dilutions. The antigen unit, by which is meant the smallest amount of antigen that gives complete inhibition of hemolysis with about two units of homologous immune serum, has been obtained by testing decreasing amounts of antigen (0.25, 0.2, 0.15, 0.1, 0.05, 0.025 c.c.), with 0.01 c.c. of serum. The anticomplementary property of the serum has been tested in 0.04 c.c. and 0.02 c.c. of undiluted serum. The antibody unit, by which is meant the smallest amount of serum that gives complete inhibition of hemolysis with about two units of homologous antigen, has been obtained by testing decreasing amounts of serum (0.02, 0.01, and of a ten per cent. dilution 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01 c.c.), with about 0.1 c.c. of antigen.

The work on the differentiation of meningococcus strains was begun with neither antigen nor serum standardized. A polyvalent antigen of all the strains was made and standardized with 0.01 c.c. of serum from a horse immunized against all the strains. The polyvalent horse serum was then titrated with one and one half units of the polyvalent antigen and used in standardizing all the monovalent antigens. Each monovalent antigen was titrated in several dilutions to obtain the antigen unit. From one and one half to two units of the monovalent antigen thus standardized were used in standardizing its homologous immune serum. Any antigen so poor that double this amount was anticomplementary was discarded and not used for serum titrations. Serum was also titrated in a sufficient number of dilutions (1 in 10, and 1 in 100) to determine the antibody unit. From one and one half to two units of serum (diluted so that 0.01 c.c. contained that amount) were used in making the antigen cross-titrations. The specificity of the antigens was tested by titrating against a known positive gonococcus serum with practically negative results.

Rabbits were immunized against forty strains of meningococcus, but the cross-fixation work was carried out completely with only twenty-nine of these strains. Each antigen and each serum were titrated against all the strains in several dilutions until the smallest amount of both antigen and serum that gave complete inhibition of hemolysis was determined.

The results of antigen titrations are summarized in the table.

Of the twenty-nine strains of meningococcus tested, fourteen fell into one group, eight into another, two cross-fixed with each other only by both antigen and serum titrations, two did not fix with any other strain and three acted irregularly. Strains 1, 3, 4, 7, 8, 19, 20, 21, 22, 23, 24, 26, 28, and 34 cross-fixed with each other, forming Group I (Tables I and II). Strains 9, 10, 11, 13, 14, 16, 18, and 32 cross-fixed with each other, forming Group II (Tables I and II). Antigens of Group I did not fix

TABLE I

Antigens of Twenty-two Strains Titrated Against Sera of Various Strains

Antigen Strains	Immune Sera of Group I									Immune Sera of Group II						Immune Sera of Irregular Strains			Immune Sera of Odd Strains			
	1	3	4	7	20	22	23	24	28	10	11	13	14	16	18	2	12	27	6	29	15	17
1	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
8	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
19	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
21	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
23	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
26	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
28		+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
34		+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
9	−	+	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	±	−	−	−	−
10	−	+	−	−	−	−	±	−	+	+	+	+	+	+	+	−	−	±	−	−	±	−
16	−	+	−	−	−	−	−	−	±	+	+	+	+	+	−	−	−	−		−	−	−
18	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	+	−	−	−	−
32	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−
2	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−
27		+	+	+	−	+	−	−	+	−	−	−	−	−	−		−	+	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+

+ Denotes complete inhibition of hemolysis in as small an amount of antigen with heterologous as with homologous serum.

± Denotes complete inhibition of hemolysis, but not in so small an amount of antigen with heterologous as with homologous serum.

— Denotes incomplete or no inhibition of hemolysis.

any sera of Group II, but antigens 9, 10, and 16 of Group II fixed some sera of Group I. Antigen titrations only of strains 21 and 26 were made against other single strains, as the rabbit sera of these strains lost their antibody content before serum titrations could be performed. These strains were not included

TABLE II

Immune Sera of Twenty-four Strains Titrated Against Various Antigens.

Serum Strains	Antigens of Group I											Antigens of Group II						Antigens of Irregular Strains			Antigens of Odd Strains		
	1	3	4	7	8	19	20	22	23	24	28	9	10	13	14	16	18	2	12	27	6	29	15
1	+	+		+	+	±	±	+	±	±	±	−	−	−	−		±	−	±	−	−	−	−
3	+	+	+	+	±	±	+	+	+	+	+	+	+	+	+		±	+	±	+	−	−	−
4	+	+	+	+	±	−	+	+				−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	−	−	+	+	+	+		−	−		−		−	−	−	−	−	−	−
8	+	±	+		+		+	+				−	±	−	−		−	±	±	−	−		−
19	+	+	+	+	+		+					+	+		−		+	±	−		−	−	−
20		+	+	+	+	−	+	+				−	−	−	−	−	−	±	−		−		
22	+	+	+	+	±	±		+				±	±	±	±		±	±	±		−		
23	+	+				+	+					±	±	±			±				−	−	
24	+	+		+		+	+					+	+				±		+	+	−	−	
28	+	+		±				+				+	+		−		−	+			−	−	−
9	±	±	±	±	±			±	±			+	+	+	+			±	±		−		
10	±	+		±	±			−				+	+	+	+	+	+	±	±	−	±	−	
13	±	±	±	±	±			±				+	+	+				±	±		±		
14	±	±	±	±	±		+	±				+	+	+	+	+	+	±	±	+		−	−
16	±	±	±	−	−	−		±				+	+			+	+	−	−		−		
18	+	+		+	+		−					+	+			+	+	−	+		−		−
2	−	−	−		−	−		−				−	−		−	−	−	+	−	−	−	−	−
12	±	±	±		±							±	±		−			±	+		−	−	±
27	±	−	±	−			±					±	±			±	±	−		+	−		−
6	±	−	−	−	−	−	−	−	−	−	−	−	−	−				−	−	−	+	−	±
29	−	−	−	−			−					−	−		−		−	−	−		−	+	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−	+
17	±	±	±	−	−	−	−	−	±	−	−	−	−	−	−		−	−	−	−	−	−	+

+ Denotes complete inhibition of hemolysis, in approximately the same amount of serum with homologous and heterologous strains.

± Denotes complete inhibition of hemolysis, but not in so small amount of serum with heterologous as with homologous strains.

— Denotes incomplete or no inhibition of hemolysis.

in an antigen made of six strains (3, 21, 10, 18, 2, and 12). The sera of these strains (21 and 26), however, gave fixation with this polyvalent antigen and the antigens of these strains fixed all the sera of Group I, hence, we concluded that these strains belonged to Group I. Antigens of strains 6 and 29 gave fixation with their homologous sera only and sera 6 and 29 gave fixation with their homologous antigens only. Antigens and sera of strains 15 and 17 cross-fixed with each other. Strains 2, 12, and 27 acted irregularly. In the antigen titrations of these strains cross-fixation occurred with the sera of Group I, but the immune sera of strains 2, 12, and 27 fixed complement with their homologous antigens only.

Sera of twelve strains (1, 3, 4, 7, 8, 19, 20, 21, 23, 24, 26, and 28) of Group I, seven strains (9, 10, 11, 13, 14, 16, and 18) of Group II, the irregular strains 2, 12, and 27, the odd strains 6, 29, 15, and 17, and nine unidentified strains (31, 32, 33, 34, 36, 37, 38, 39, and 40), were titrated with a polyvalent antigen consisting of strains 3 and 21 of Group I, 10 and 18 of Group II and the irregular strains 2 and 12. The relationship of strains 6, 15, 17, 27, and 29 had not been determined at the time this antigen was made or they would have been included. These strains only gave no fixation with the antigen, but our later work demonstrated that they were not related to any of the strains included in the antigen.

As stated above, the monovalent antigens were standardized with the polyvalent immune horse serum for the serum cross-titrations, which were made before the antigen cross-titrations. We found after we had done the antigen cross-titrations, that the antigens gave a much longer range of fixation with their homologous immune rabbit serum than with the polyvalent horse serum. It seems, therefore, that so much antigen was used in the serum cross-titrations that a group reaction occurred. This may explain why a more distinct differentiation of strains was obtained by means of antigen titrations than by means of antibody content titrations.

Complement fixation is such a delicate method of differentiating strains of an organism that it is difficult to obtain absolutely consistent results. The age and antibody content of a serum, the range of the antigen, the strength of the complement, the interval between the completion of the test and the reading of the same, the temperature of the ice-box, and the frequency of standardization of sera and antigens are factors that greatly influence the test.

A study of the history of the strains proves that the clinical history of the case has nothing to do with the grouping.

Strains causing severe reactions in rabbits—it being necessary to try several rabbits before one could be found that could be immunized—were:

1, 2, 7, 16, 18, and 24,—
1, 7, and 24 are in Group I,
16, and 18 are in Group II, and
2 in irregular Group.

Thus it appears that no relation exists between virulence and grouping. Neither is there any relation between length of time strains had been isolated and grouping. Group I contained the oldest and the newest strains.

The subject of the para-meningococcus has received considerable attention in France for the last few years, due to the work of Dopter, Menetrier and others. This work has been done along both clinical and laboratory lines. Patients suffering from meningitis that did not respond to the ordinary anti-meningitis serum improved under the use of a para-meningitis serum prepared by Dopter in 1912. Dopter in 1911 in studying the so-called para-meningococcus, found that he could not differentiate these strains from normal meningococcus by using them as antigens with polyvalent horse serum, but that they could be differentiated by the serum of patients, as the serum of patients suffering from meningococcic meningitis did not fix a para-meningococcic antigen, and the sera of patients suffering from para-meningococcic meningitis did not fix with a meningococcic antigen.

Wollstein in 1914 published a very careful study of the para-meningococcus in which she arrived at similar conclusions using the sera of rabbits immunized with single strains of meningococcus and para-meningococcus instead of the sera of patients. She concluded: "The para-meningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation."

By the agglutination method strains 7 and 42 are classed by her as para-like strains. She did not give the results of complement fixation with these strains. In our work it was found by complement fixation that strain 7 fell in Group I and strain 42 in the irregular group allied to Group I. As transplants of two Dopter para-meningococcus strains, P. L. and P. M., were kindly given us by Dr. Wollstein, we were enabled to test them against our strains. We found antigens of P. L. to react with homologous serum only, and antigens of P. M. to react with the sera of Group II.

Now if the para-meningococcus is to be separated from the normal meningococcus by serological reactions and if agglutination and complement fixation yield different results with the same strains, the question is indeed a perplexing one. With the method used by us, the results yielded by the complement fixation test have been fairly uniform and specific. By this method the strains have fallen into various groups, containing a varying number of strains. Shall we call all the strains except those in Group I para strains, or shall we call them simply groups of normal meningococci? The question is purely academic, yet it is of some importance since the employment of the term "para" differentiates the strains more than seems justifiable. It is perhaps better, therefore, to classify them as belonging to a group co-ordinate with other meningococcus groups.

Wollstein further concludes: "Because of the variations and irregularities of serum reaction existing among otherwise normal strains of meningococci it does not seem either possible or desirable to separate the para-meningococci into a strictly definite class.

It appears desirable to consider them as constituting a special strain among meningococci not, however, wholly consistent in itself." This conclusion we would heartily endorse.

CONCLUSIONS

By means of complement fixation the meningococcus may be clearly differentiated from allied organisms. A differentiation of individual meningococcus strains is possible by the use of refined technique, but the relationship of strains is so close that it is difficult to obtain absolutely clear cut and consistent results. Of the twenty-nine strains studied by us, fourteen seem to form one group and eight a second group. Three seem to be closely related to the first group but have acted so irregularly that they cannot be classed with it, two have shown a relationship with each other only and two have shown a relationship to no other strain.

Discussion:

Dr. Zinsser: The difficulty of separating and classifying the various strains of meningococci is well known. This is the first serious attempt to clearly separate the different strains by complement fixation tests.

THE PRACTICAL SIGNIFICANCE OF THE OXIDASE REACTION AS APPLIED TO BLOOD CELLS

F. A. EVANS, M.D.

For some time it has been known that the cells of myeloid origin contain granules of oxidase ferment and those of lymphoid origin do not, and an appreciation of the practical significance of this difference has led to many attempts to perfect a convenient method of demonstrating the presence or absence of the ferment in any given cell. As early as 1887, Vitali made the observation that the guaiac test for blood was of no value in the presence of pus, but Brandenburg[1] in 1900, was the first to notice that blood of leukemic patients frequently gave a positive guaiac reaction without the presence of any oxygen bearer as peroxide or ozonized turpentine. Meyer[2] in 1903, demonstrated this phenomenon in the blood of myeloid leukemia but failed to obtain the reaction in that of lymphoid leukemia and called attention to the interest that attached to this reaction in view of Ehrlich's recently propounded dualistic theory of blood cell origin. This guaiac test was too crude to be of any practical value however, so Winkler[3] in 1907, applied the indophenol-blue synthesis of Ehrlich[4] (alphanaphthol + dimethylparaphenylendiamin with two atoms of oxygen form indophenol-blue) to smears of pus and tissue sections and with it found these granules of oxidase ferment as blue-black dots in the protoplasm of myeloid but not of lymphoid cells. He at this time predicted the usefulness of this reaction in the study of the origin of white blood cells, and it is the practical value of this test that we have under consideration this evening.

Schultze[5] submitted sections of many body tissues to this reaction after formalin fixation and found the granules only in the tear and thyroid glands and in cells of myeloid origin, and used the test in the differential diagnosis of the leukemias. Gierke,[6] using fresh tissue, found these granules in many body cells but after formalin fixation as used by Schultze obtained similar results. He suggested the hypothesis that this reaction on fresh tissue depended on the circulating respiratory oxygenase destroyed or inactivated by formalin fixation whereas the more stable oxidase of the myeloid cell endures. This work of both Schultze and Gierke has been repeatedly confirmed by other observers.[7] Gierke also refuted Dietrich's[8] statement that the oxidation is brought about by the tissue metabolism and the preformed indophenol-blue taken up by the fat droplets, by showing that no granules appear after killing the ferments and adding the already oxidized solutions, and that granules still give the reaction after killing the cell life by chloroform but preserving the oxidases. This indophenol-blue reaction appears, therefore, to be a reliable method, but although much superior to the guaiac test, because of technical difficulties is not all that is to be desired.

For fixation, only methods are available that do not injure ferments and as shown by Gierke,[6] formalin must be used in some form. Similarly counterstaining is difficult for only aqueous solutions used without a mordant are permissible, and any method of differentiating destroys the reaction in some cells. Furthermore, inasmuch as this indophenol-blue deposited in the granules of oxidase ferment is soluble in alcohol, ether, chloroform, aceton, xylol, and creosote, clearing of tissue sections is impossible and the mounting media are almost limited to materials soluble in water. Also, the preparation is not permanent, for the oxidase reaction only endures in its original intensity for about an hour, and the counterstain tends to be dissolved out by the aqueous mounting medium.

In spite of these difficulties, methods of application have been devised that are of practical value for tissue sections and especially for smears of blood and body fluids. For tissues, paraffin sections may be used if fixed primarily in formalin or preferably Bonn solution (Carlsbad salts 5, formalin 12.5, water 100 parts) as suggested by Fursenko,[9] for the materials used in preparation presumably do not injure the ferments. Frozen sections freshly cut give reliable results more constantly and are to be preferred. The section is immersed for about two minutes in a mixture in equal parts of the reagents freshly made up:

(1) 1 per cent. alpha-naphthol in 1 per cent. KOH.

(2) 1 per cent. aqueous dimethylparaphenylendiamin.

These solutions should be mixed just before using for if they are allowed to stand together for any length of time they combine with the oxygen of the air with the formation of indophenol-blue and confusing artefactions are likely to occur if granules of this preformed indophenol-blue are deposited on the section. The tissue removed from this mixture is washed, counterstained for a few moments in 5 per cent. pyronin or saturated aqueous safranin, washed again, and mounted in glycerin or a sugar mixture. Such a preparation shows the nuclei stained a deep orange-pink and the myeloid cells standing out by reason of the blue granules in their protoplasm. However, owing to the im-possibility of clearing the section satisfactorily and the somewhat indefinite counterstaining, reliable identification of the individual cell as polymorphonuclear, myelocyte, or other, is frequently difficult. For this purpose parallel sections must be stained with hematoxylin and eosin and corresponding fields compared; or the field showing oxidase-containing cells may be drawn with a camera lucida, and the same section then stained with hematoxylin and eosin. If a frozen section has been used, after removing the cover slip and washing, the section is best dried and fixed to the slide with thin celloidin solution. Without these auxiliary procedures, the reaction is of value in demonstrating mild grades of infection in cellular tissues as the appendix and tonsil, and in demonstrating myeloid infiltration of organs in leukemia; and with them may be used to advantage in studying any obscure body cell. Many modifications of the indophenol-blue reaction have been suggested by Loele,[10] Schultze,[11] Kreibich,[12] and others,[13] whereby permanent preparations that may be cleared and counterstained may be had, but in all of these so many other factors are to be considered that their specificity for the myeloid cells may be seriously questioned. These modifications, although of great interest in the field of vital staining,[14] do not displace the original indophenol-blue reaction.

For smears of blood and other body fluids a satisfactory and more direct technique is available. Fixation is the most important step and for this, exposure to formalin vapor in a closed jar is the only satisfactory method. Very weak solutions of formalin (4 per cent.) for long periods (3–4 hours), depending on the age and thickness of the smear, are better than using stronger solutions for shorter periods, for with the weaker solutions the annoying tendency of the fixed smear film to wrinkle up when the indophenol-blue solutions are added, is not so marked. For smears, in contrast to tissue sections, the staining is best done first and for this the same solutions, 5 per cent. pyronin or saturated safranin, have proved the most satisfactory. Either of these allowed to remain on the smear a short time and then thoroughly washed off brings out the nuclei a deep orange-pink that readily admits of the identification of the cell and leaves the other elements unstained. Such a preparation is then examined until a field containing cells in question as regards their oxidase content is found, and the slide clamped down. One drop of each of the solutions of the indophenol-blue reaction made up as outlined above is then added directly to the smear and the progress of the reaction watched with the low power objective in the cells being investigated till it has reached its maximum intensity in about two minutes. The smear may then be blotted carefully and the preparation examined with the high dry objective. It is essential that the cells in question be located before putting on the solutions of the indophenol-blue reaction and that the progress of the reaction be watched, for the stain is rapidly dissolved out of the nuclei by the alkaline solutions used and shortly after adding, especially if some wrinkling of the film as frequently occurs is present, identification of the cells becomes impossible. But if these details are observed a useful preparation results which shows oxidase granules in polymorphonuclears, myelocytes, myeloblasts, transitionals, and probably some large mononuclears, but in no lymphocyte. As in tissue sections the reaction is of value in the study of cells of uncertain identity and is especially useful in the differential diagnosis of the acute leukemias which is so difficult by the usual staining methods. The intensity of the reaction varies directly with the stage of maturity of the cells, being weakest in the myeloblasts and strongest in the polymorphonuclears. One observer[15] suggests that granules of oxidase ferment may not be demonstrable in very young myeloblasts. The eosinophile cells both in blood and tissues show the most active oxidases.

Owing to technical difficulties the indophenol-blue reaction falls somewhat short of the promise it gives at first thought, and modifications devised to obviate these difficulties are not trustworthy. But the methods outlined above, if followed in detail, afford a reaction on tissue sections that is of some value, and one on blood smears, that by reason of the ready differentiation between cells of lymphoid and myeloid origin made possible, is of real diagnostic importance.

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Discussion:

Dr. Lambert: In two cases of myeloblastic leukemia recently observed in the Presbyterian Hospital, this reaction proved very valuable. Under casual examination I am certain that the cells in the blood would have been called lymphocytes. In fact, this mistake was made. The oxidase test proved conclusively, however, that the cells were of myeloid origin.

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DEMONSTRATION OF THREE CASES OF SPLENIC ENLARGEMENT

MORTIMER WARREN, M.D.

These three specimens represent different types of splenic enlargement, and are presented to bring up the question of clinical differentiation, and pathological classification of splenomegalia in general, with reference to therapeutic splenectomy.

CASE I.—The spleen is from a patient presenting a well-characterized clinical condition with striking differential diagnostic signs, namely constitutional hæmolytic icterus. This spleen was removed by Dr. Charles H. Peck in May, 1912. A complete symptomatic cure resulted, namely relief of jaundice, and a reversal of a previously highly decreased osmotic tension of the red blood cells to one within normal limits. Histologically it is typical of that condition. The spleen is suffused with blood; this blood is chiefly interstitial; there is little or no fibrosis; follicles are somewhat crowded out. In other words, it is a spodogenous spleen tumor very similar to that of pernicious anæmia.

Case II.—The spleen represents a type which has many designations but which seems to belong to the Banti group. Though still "*sub judice,*" as an entity, Banti's disease has a place in any discussion of splenic enlargement. There is a group of cases in which clinical results are obtained by splenectomy which cannot be listed elsewhere except under the general terminology of "splenic anæmia." This spleen shows marked diffuse fibrosis without a great deal of capsular thickening, obliteration of follicles, and thickening of arterioles. There is little blood or congestion. (A small piece of liver removed at the time of the operation appears normal on section.) Splenectomy was performed by Dr. Charles H. Peck, March, 1914. The man was in good condition when last heard from, within a few months of the present time.

Case III.—The patient showed an enlarged liver and spleen, jaundice, urobilin and bilirubin in urine, and clay-colored stools. There was nothing important in the blood findings aside from the auto-agglutinating property of the blood serum for the red blood cells (brought out only in the cold). Splenectomy was done by Dr. Charles H. Peck, September 15, 1915. Results so far are indefinite. The spleen shows wide open sinuses, areas of distended capillaries, abundant follicles, fibrosis of a fairly young character (cellular). The spleen looks as if it were in a hyperactive state, analogous to a lymph node in a state of irritative hyperplasia. A small bit of liver removed at the time of operation showed inflammation of a subacute character. There are periportal and scattered areas of round cell and polynuclear infiltration with some peri-portal increase of connective tissue. A diagnosis of peri-portal pylephlebitis was made.

I should like a discussion, especially in regard to the condition of the spleen accompanying cirrhosis of the liver, the spleen of malaria, and of syphilis.

Discussion:

Dr. Norris: These cases are of great interest. Much importance has been attached to the spleen in regard to its association with various diseases. I do not feel able to give a competent opinion upon the lesions of the spleen. It has always been a subject of great dispute among pathologists. Even the histology of the spleen has not been definitely settled. The lesions that Banti has described as characteristic for the disease known by his name, namely, fibro-adenia, I have found frequently present in the spleen where Banti's disease has not been present, namely, in association with many of the conditions found in middle life and also with recessive status lymphaticus. It is quite frequent to find thickened or nearly obliterated arteries of the splenic follicles, and often there are marked fibrous changes not only about the vessels but in the stroma. I cannot say, therefore, that these changes are characteristic of any one disease and therefore not characteristic of Banti's disease. A number of pathologists have described the spleen as presenting characteristic appearances not only in the gross but on microscopic section; this has not been my experience. In syphilitic cirrhosis of the liver, the enlargement of the liver is often marked and it is well known that the size of the spleen apparently bears no especial relation to the degree of the cirrhosis of the liver.

DR. LARKIN: We are in a hopeless condition, it seems to me, in regard to the classification of splenic lesions. I feel Dr. Norris is right. There are so many allied conditions which bring enlargement of the spleen which have no special characteristics, that we are hopelessly involved. Dr. Warren's specimen shows well marked enlargement and engorgement of the spleen in the thrombosis of the splenic vein. We do not know any more than we did many years ago about the exact condition of the circulation of the spleen, histologically.

DR. LEVY: What was the red cell count in these cases?

DR. WARREN: None of the three cases showed any anemia of any account.

DR. LEVY: Was there polycythemia?

DR. WARREN: No.

DR. ZINSSER: Did I understand Dr. Warren to say that one of these cases had hæmolysins for its own cells?

DR. WARREN: There were auto-agglutinins in the third case brought out only in the cold.

DR. ZINSSER: These conditions seem to be analogous to paroxysmal hæmoglobinuria.

DR. WARREN: I don't know what it means. There did not seem to be direct clinical indication for operation, except on the basis that the spleen had an important bearing on the liver condition and might keep up the disturbance in the liver.

DR. ZINSSER: In paroxysmal hæmoglobinuria an autolysin is found which does not act under the ordinary conditions of incubation. Hæmolysis occurs only when the sensitization has occurred at a low temperature and the tubes are consequently warmed up. I should like to ask whether there are any described splenic conditions associated with paroxysmal hæmoglobinuria.

DR. WARREN: I don't know. In these jaundice cases it is associated with the bile production.

WASSERMANN FINDINGS IN LOBAR PNEUMONIA

J. J. HERTZ, M.D.

My communication this evening is a preliminary report on the Wassermann findings in lobar pneumonia. At the Beth Israel Hospital where we do a Wassermann reaction on every patient admitted we found in a series of twelve cases the following results:

I. Ten of the twelve cases got well.

II. Every one of the ten cases that got well gave a "four plus" Wassermann reaction during the course of the disease, between the fifth and eighth day, and a negative reaction after the temperature became normal.

III. Of the two cases that died, one gave a "four plus" reaction on the sixth day and died on the seventh day. On autopsy the organs showed signs of syphilis. The other case that died gave a negative reaction on the eighth day about two hours before death. The only negative case died.

We are carrying on some experiments to find the nature of the substance that gives the reaction in these cases.

We used the original Wassermann test with the exception of the antigen which was an alcoholic extract of guinea pig's heart.

TRANSPLANTABLE SARCOMATA OF THE RAT LIVER ARISING IN THE WALLS OF PARASITIC CYSTS[1]

G. L. ROHDENBURG, M.D., AND F. D. BULLOCK, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

Primary sarcomata of the liver, while not common in man, are relatively frequent in rats, probably because of the relation they bear to infection with the *Tænia crassicola.* In a series of autopsies on rats performed during the past three years, we have encountered three such tumors, two of which were recognizable in the gross, while one was of microscopic size only.

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[1] This report appears in full in *The Journal of Cancer Research*, 1916, I, 87.

The first case has already been reported.[2] The second case occurred in a full-grown male, the tumor being situated in the left lobe of the liver and measuring one centimeter in diameter; metastases were scattered throughout the peritoneal cavity. The neoplasm, a spindle-cell sarcoma, is transplantable, and is now in the tenth generation, with an inoculation percentage of 100. About eighty per cent. of the takes retrogress spontaneously. Attempts to propagate the tumor by using a Berkefeld filtrate and dried tumor tissue were not successful. The third case occurred in an adult female; the tumor was situated in the Spigelian lobe and measured 1.5 cm. in diameter, and there were peritoneal metastases. This tumor also is transplantable, though the percentage of takes is not as high as in the case of the second tumor.

The chief point of interest in these cases is the demonstration of the parasite in the centers of the tumors.

Discussion:

Dr. Zinsser: I would like to ask whether it was at all possible to see where the tumor started.

Dr. Rohdenburg: The tumors started in the cyst wall surrounding the parasite.

Dr. Zinsser: Does the cyst wall originate from the capsule of the liver? Can the cysts be experimentally produced in rats?

Dr. Rohdenburg: Yes, the rats can be infected with the tenia and cysts produced in this way.

Dr. Norris: Was the pancreas tumor an original tumor or a transplant?

Dr. Rohdenburg: It was a metastasis of the original tumor in the pancreas.

Dr. Zinsser: Is the percentage of tenia findings in rats very high; that is, is the percentage of tumors large or small as compared with the percentage of tenia infection?

Dr. Rohdenburg: The presence of the tenia in rats depends chiefly upon the condition in which the dealer keeps his animals. If he changes the drinking water frequently tenia infection is rare; if not, it is common. The tumors occur in only a small percentage of the infected animals.

Dr. Norris: I would like to ask exactly what the sarcomatous process was in the transplants; in other words, what method of injection was used in the transplants.

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[2] *Jour. Med. Research,* 1913, XXVIII, 477.

Dr. Rohdenburg: The material used in the first case was the healthy part of the tumor, about 0.5 cm. distant from the parasite. In the second case, the metastases were used for transplantation.

Dr. Zinsser: You never had metastases in the experimental tumors?

Dr. Rohdenburg: No, not in the transplants.

Dr. Norris: In other words, it is doubtful whether we can call the original tumors sarcomata?

Dr. Rohdenburg: If a malignant tumor can be defined as a collection of cells showing unlimited powers of division, I do not see why the primary tumors should not be called sarcomata. Even the fact that some of the transplanted tumors receded is not an argument against their sarcomatous nature. The Jensen sarcoma recedes in thirty out of fifty transplants; but some of the transplants grow, and produce metastases.

A CASE OF PORTAL THROMBOSIS WITH UNUSUAL COMPLICATIONS

JOHN H. LARKIN, M.D.

Thrombosis of the portal vein is not an unusually rare condition. This will be seen after a perusal of Lissauer's report in *Virchow's Arch.*, 1908, CXCII, 278, which states that among 26,687 cases coming to autopsy a condition of complete or partial occlusion of the portal vein was found sixty-eight times. His very interesting resumé also gives us an idea of the duration of such thrombi in the portal vein and its tributaries, together with the concomitant lesions, such as circulatory ileus, apparent in the most advanced cases. Numerous writers have contributed largely to the pathology of this condition, among whom we might mention Welsh, Versé, Rosenau, Pick, Heller, Lewis, Flexner, Whipple, Winternitz, Kohler, and Chiari. It is now well known that there is a close etiological relationship between portal thrombosis and bacterial infection, and this has been especially dwelt on by Welsh. The primary changes in the intima of the portal vein, together with a slowing of the blood stream, as well as compression of the vein by thickening of its adventitia, pressure of large lymph nodules in the vicinity of the hylus of the liver, or inflammatory adhesions to the wall of the vein are etiological factors which have been well pointed out by many observers. Its relationship with metastasizing tumor, either in the liver itself or in distant parts, blocking the vein with such growth, either directly or by extension into the vein itself, has not been so often referred to in the literature of this condition.

It is interesting to note that in all the cases of portal thrombosis which have been reported the liver itself suffers very little secondarily from such circulatory disturbances as would lead on to infarction.

I wish, however, to present a condition of thrombosis, the etiology of which is foreign to our ordinary conception of the disease. It presents a number of factors of great clinical significance, and on account of the close clinical picture of the individual during life with that of cirrhosis of the liver, I trust that the presentation will be of interest to all.

The history of the individual is as follows:

A negro; forty-five years of age; admitted to City Hospital on February 17, 1915. Died March 30, 1915. Chief complaint was swelling of legs and shortness of breath. Family history: Father died at eighty years, of senility; mother died at sixty-four, cause unknown. Patient was one of fifteen or sixteen children, some of which died in infancy, but the majority are living and well. There was no tuberculosis, cancer, alcoholism, heart, kidney, or venereal disease elicited in the family history. Personal history: Had the ordinary diseases of childhood: measles, diphtheria, typhoid fever, etc. No history of venereal disease obtainable. Occupation was that of a private chef in one of the clubs, where he had been employed for a number of years; he was a mild drinker of beer and whiskey. Three and one half or four years ago patient had an axillary abscess, which was opened.

Last July, while in apparently the best of health, in helping remove a trunk he was thrown down a flight of stairs, a distance of some twenty feet, striking on the trunk at a point on his abdomen midway between the ensiform and the umbilicus in the median line. Upon being picked up and examined it was found that a small hematoma had developed in the rectus muscle, about five cm. below the ensiform. This hematoma was from five to six cm. in diameter. It was not accompanied by any unusual pain at the time, except for skin tension. He was advised by his physician to go home. Late on the same evening, around nine o'clock, he commenced to complain of lancinating, shooting pains in his abdomen, accompanied by a partial collapse and vomiting, the vomitus being a yellow, bile-colored fluid. The pain at this time became so severe that opiates had to be administered. On the following day the patient was no better. He remained in bed for a period of two weeks, during which time the pain had diminished in severity, being deep-seated in character.

In the beginning of August the patient's legs became quite markedly swollen, and in one of his attacks of vomiting he had a hematemesis of bright red blood, jelly-like in consistence and about four ounces in quantity. He was treated by his physician for gastric ulcer.

About the beginning of October his abdomen commenced to swell, but not to such an extent as to make him feel uncomfortable. Swelling of the legs continued and shortness of breath was marked at this time. Examination of his abdomen gave fluid wave by percussion. Patient seemed rather anxious in his manner, and commenced to be emaciated. His color was a light yellowish, which was most marked in the conjunctivæ.

About November 1, patient had a very large gastric hematemesis, losing about a quart of clotted, jelly-colored blood, and at about this time it was noted that his stools were dark and tarry-colored, evidently occult blood. From this time on the patient seemed to become more and more depressed. The swelling of the abdomen became larger and larger, and by the first of December fluid had accumulated to such an extent that it was deemed necessary to tap the patient in order to relieve the fullness of his abdomen. A very large quantity of straw-colored fluid was obtained at this time, after which the patient went into a collapse. Soon after another large hematemesis occurred, which contained many jelly-like clots. The patient's condition at this time was that of a fretful and worried individual. The deep-seated pain now seemed to have disappeared.

The patient remained quietly at home until the first part of February, when he was removed to the City Hospital. Upon admission the patient was found to be a fairly well nourished individual. His abdomen was swollen and both legs were moderately edematous. He complained of a dull, deep-seated pain in his abdomen; his expression was anxious, and he was short of breath. The anemia of the individual was well marked, denoting great loss of blood. On the day of his admission, Feb. 17, patient had another large gastric hematemesis. His stools were tarry-black in color. From this time on until March 30, the patient remained in bed, complaining little, except of abdominal pains and shortness of breath; also of an increasing distress on account of abdominal fluid. On March 10, patient complained so much that tapping was resorted to, and a large amount of straw-colored fluid was withdrawn. On March 27, patient had another large gastric hematemesis, which was repeated on the following day. On March 29, the patient was found in a very weak condition; the abdomen was again commencing to distend. On March 30, patient refused nourishment. He had a final hemorrhage of a large amount of blood containing many clots, and died shortly afterwards. An autopsy was performed on the following day.

The autopsy findings were as follows: Body is that of a fairly well developed colored man about forty-five years of age. There are no physical abnormalities upon external examination.

Heart weighs 500 gm. The left ventricle is dilated. Muscle is pale, friable and flabby. Width of the aorta about the aortic valve is 7 cm. Depth of ventricle is 7 cm. Thickness of the wall of left ventricle is 1.5 cm. While the aorta is slightly thickened, its intimal surface is smooth, except for several slightly raised nodulations, mostly of a fatty atheroma. The pulmonary orifice measures 9 cm. Tricuspid valve measures 13 cm. Mitral valve measures 9 cm. Foramen ovale is closed. Aortic valves are competent; they are slightly thickened along their free edges. The mitral, tricuspid and pulmonary valves show no lesions.

Left lung weighs 500 gm. Organ is bound down in the pleural cavity by adhesions. It is voluminous; contains about the normal amount of pigment. Mucous membrane of the bronchus is pale; there is no thrombus in the pulmonary artery or veins. The lung is air-containing throughout. On section the posterior and dependent part shows edema and a moderate congestion. There is no pneumonia. On section upper anterior lobe is pale and anæmic. There is no edema.

Right lung weighs 600 gm. It is bound down in the cavity by adhesions. Lower base of right lung shows compression atelectasis by fluid in the pleural cavity.

Liver weighs 1,600 gm. Portal vein is thrombosed. Surface of organ is indented and irregular. Consistence of organ is firm. Surface is indented by deep cicatricial scars, bringing about a great deformity of the liver. There is atrophy of the extreme left lobe of liver. The capsule is thickened by an irregular veil-like thickening, which evidently is a distension of the lymphatics. On section, liver shows a rather uniform appearance. Markings are partly obliterated. There is no evidence of connective tissue. The thrombus extends even into the portal radicles. The gall-bladder is enormously distended with bile; no stones.

Spleen weighs 800 gm. Organ shows a chronic interstitial splenitis. Splenic vein at its middle and distal portion is extremely dilated, and at about the junction of the inferior mesentery and the splenic vein there is an aneurismal dilatation at that point in the vein in which there is a parietal, organized thrombus. This has extended up into the portal vein, completely occluding its lumen. It is glistening-white in color, and shows partial canalization.

Pancreas shows extreme atrophy, the normal pancreatic tissue being replaced by fat. There is a supernumerary pancreas.

Left kidney weighs 350 gm. It measures 11 × 6 × 3.5 cm. It is uniformly glazed, and has the appearance of amyloid. The organ does not, however, react to iodin. Markings are obliterated; organ is elastic; has an almost stony hardness; it is not congested. The Malpighian bodies are slightly seen. Capsule of kidney is not adherent, but when pulled off diffuse cicatricial wedges of fibrous connective tissue can be seen. Organ has the appearance of a subacute diffuse nephritis.

Right kidney weighs 325 gm. It measures 11 × 6 × 3.5 cm.

Organ is enlarged; capsule strips with ease. On section it is similar to the left, except that it is slightly more reddish in color. The surface beneath the capsule is not granular. The markings of the kidney are obliterated.

Aorta is slightly thickened; its elasticity is slightly diminished; there is a moderate atherosclerosis, but fatty change in the intima is the most pronounced change. There is no calcification; no evidence of syphilitic atheroma.

Bladder is distended with urine; mucous membrane appears pale, but is normal.

Small intestines are full of blood, which has recently stained the mucosa.

Stomach is dilated and full of blood. It shows a hypertrophic gastritis.

Esophagus is unusually dilated. The venous radicles are enormously distended, and at a point 4 cm. above the pyloric end of the stomach there is an ulcerated area in the mucous membrane leading directly into the esophageal plexus, from which constant bleeding has occurred. Above, at a distance of 13 cm., are three other ulcerated areas, the largest of which is 1 mm.

Anatomical diagnosis: Thrombosis of the portal vein, extending into the portal tributaries and liver; varicosities of the esophageal vein (extreme); multiple rupture of varices; acute diffuse nephritis; chronic hyperplastic spleen; atheroma of aorta; supernumerary pancreas in the duodenum; degeneration of the myocardium; fatty infiltration of pancreas.

Upon further examination of the esophagus, four longitudinal, greatly distended varices take up the lower third of the esophagus; the largest of these measures 2 cm. in diameter. The varices are of unusual dimensions, and many punctate points, filled with thrombi, can be seen.

I thought on account of the enormity and intense dilatation of the varices that it would be a good opportunity for the study of such varices. I therefore placed a cannula in one of the lower varices and washed out with cold water the whole esophageal venous system. I then made a mixture of red oxide of lead and chalk (a mixture which is commonly used in making arterial injections in the autopsy room) and using only slight pressure, filled the esophageal varices, the fluid entering with little or no difficulty. After dissecting out the esophagus, it was placed in cold water until the following day, when an *x*-ray of the distended varices was taken, an illustration (Fig. 1) of which is herewith reproduced.

In a resumé of the literature on thrombosis of the portal vein we find that it is not unusual for this condition to be accompanied by esophageal varices, and that one of the attending clinical features is gastric hematemesis. But in a very careful examination of the literature of such cases no report of a case of portal thrombosis is disclosed which shows so enormous a distention of the esophageal varices or an illness which existed for so long a time as the case which I present to you here to-night. The only case approaching it in this respect was the one reported by Versé, and this, it should be remembered, was some ten years ago. His case is rather unique, and corresponds fairly well with the case presented to you now. Versé, however, gave no measurements of the esophageal distention, nor did he give measurements of the esophageal varices.

I think I am safe in stating that never before has a similar specimen been presented to this Society prepared as this specimen has been prepared, presenting, as it does, a clean-cut anatomical view of the esophageal varices, and showing to what a huge extent such varices may be distended.

The transverse measurement of the esophagus taken at the time of autopsy is 12 cm., and the largest varix, which is almost a venous aneurism, measures 2 cm.

Many punctate points may be seen, from which bleeding has occurred from the distended veins.

A further dissection of the organs reveals the following:

The spleen is enormously enlarged, and on section of the pancreas from tail to head one immediately encounters the distended splenic vein, which, at its middle part, measures 1.5 cm. Just before the splenic vein meets to form the portal there is a giving way of the splenic vein, and an aneurism has formed, the wall of which is now lined by a laminated and organized clot, pearly-white in color, and of firm consistence. From this point on the portal vein is entirely thrombosed, reaching up to the portal radicles. Yet upon macroscopical examination the liver is found to be negative, except that the superior surface of the left lobe seems to be indented by deep cicatricial scars, the apex of which was toward the greater bulk of the liver substance, and

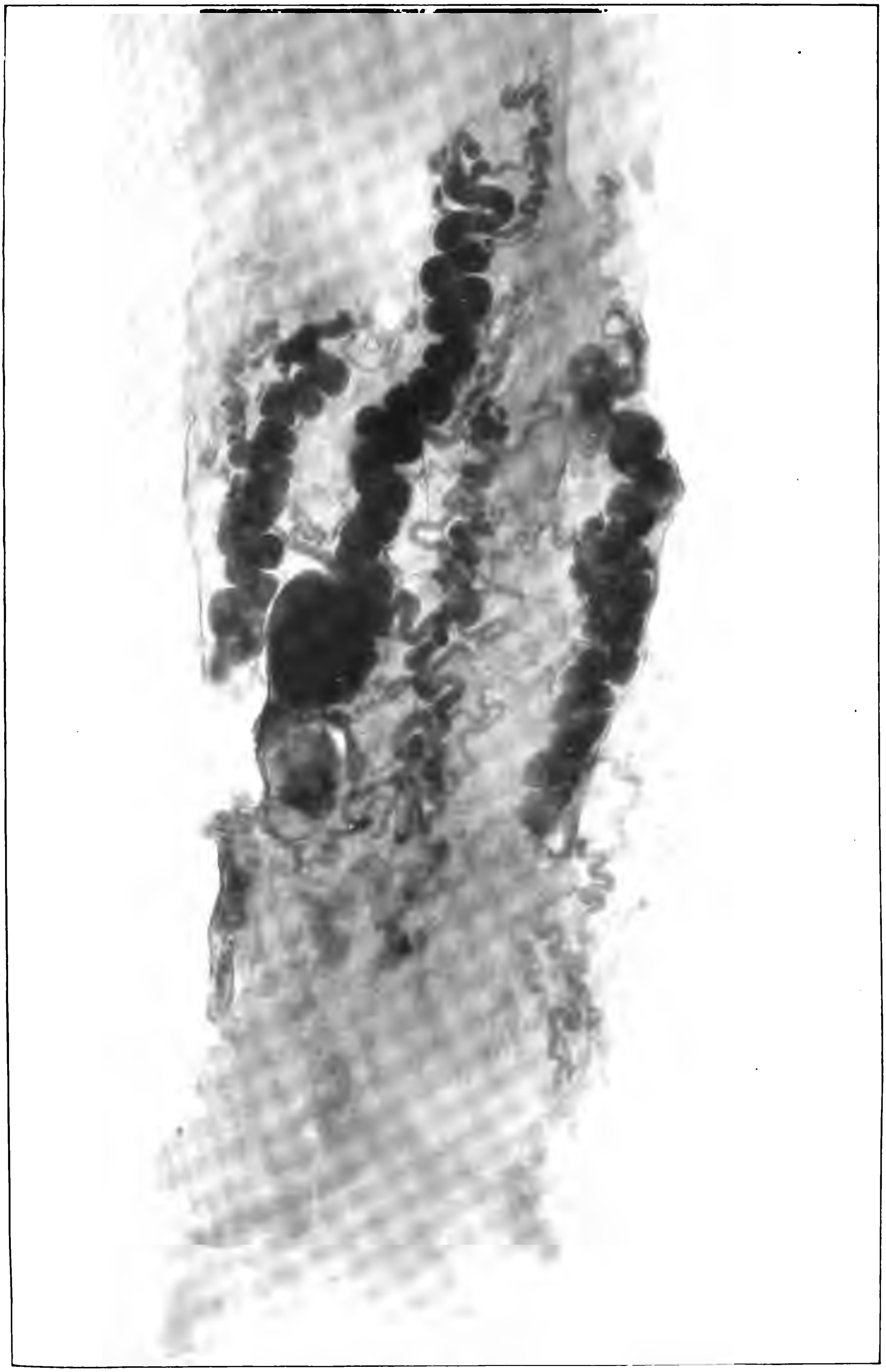

FIG. I. Excessive Esophageal Varix in a Case of Portal Thrombosis.

the base peripherally pulling down and deforming the greater part of the liver tissue. Some of these cicatrices measure 1 cm. from base to apex.

Microscopical examination of the splenic aneurism and portal vein shows a trauma, with resulting aneurismal dilatation in the splenic vein at its junction with the portal. Here the thrombus at its peripheral part is organized, the wall of the vein having given away and showing a much older pigment distributed throughout in the neighborhood of the pancreatic tissue. Examination of the portal vein shows beginning parietal organization, the central part, however, being composed of a firm mixed clot.

Microscopical examination of the liver shows no pathological changes. The cicatrices at the peripheral part of the liver capsule show large triangular areas of dense fibrous connective tissue, in which the hepatic artery and the portal radicles seem to be condensed by such fibroses. No infarction of the liver can be made out either macroscopically or microscopically, and the present interpretation of such cicatricial wedges does not now seem clear to the writer.

This case is of unusual interest, not only to the clinician but to the pathologist, by reason of the very close clinical picture exhibited in portal thrombosis, and disturbances of the portal circulation in cases of cirrhosis of the liver.

It is of far greater interest, however, on account of the etiological factor presented, which is undoubtedly one of trauma—a factor which has not heretofore been mentioned in any report of similar cases. It presents great clinical interest, too, by reason of the enormous distention to which the esophagus may be put before rupture and death intervene. The extent of this dilatation is faithfully shown in the accompanying photograph (Fig. 1).

Discussion:

Dr. Norris: This case of Dr. Larkin's is interesting. In the photomicrograph shown there was evidence of phlebitis. I don't think there is any doubt that trauma was the etiological factor in this case. I notice that the pancreas was large. The relation between latent and sub-latent infections of the pancreas and appendix is interesting. The veins of these organs drain into the portal vein. In view of the trauma in this case, with the damaged vein, there probably developed a lesion of the portal vein which gave rise to thrombosis. In regard to the esophageal dilatation, the thrombus must have been acute and the veins were not able to take care of the distention.

DR. LARKIN: I am glad to hear Dr. Norris has agreed with me in the view of trauma as an etiological factor. I have looked over this subject very thoroughly. In all the cases that have come under my observation the question of trauma has never arisen. I have asked a number of well-informed clinicians but have had no agreement on the etiology of this condition.

BILATERAL INFARCTION OF THE SUPRARENAL BODIES

JOHN H. LARKIN, M.D.

The specimen of infarction of the suprarenal bodies presented to you this evening is so very rare that it deserves more than passing notice. There has been no case similar to it reported in medical literature, save a meager resumé of a unilateral thrombosis of the suprarenal, described by Paul G. Woolley in the *Journal of Medical Research,* 1902. That was a case of an eleven-months-old child, in whom he had found a thrombus of the right adrenal vein, with resulting engorgement and infarction. It is quite well known that hemorrhages in the suprarenal bodies may be unilateral or bilateral, and may be found at the time of autopsy in newborn infants, but to this I do not refer.

The history of the case presented here tonight, in keeping with the findings, is very unusual.

The patient was a young man twenty-four years of age, a muscular iron-worker. He had worked until his admission to the City Hospital on June 16, 1915. His first symptoms were those of pain in the abdomen, in the region of the ensiform. The pain was of a lancinating character, so that opiates had to be administered to control it. The patient's personal and family history were both negative. He was married, and had one child; he denied the use of alcohol and tobacco. A Wassermann test made at time of admission gave a negative result. Temperature upon admission was 101°. A blood culture taken the same day gave negative findings. Patient was hard to control; he wanted to wander around the ward; at times he was doubled up with pains in the abdomen, crying that something be done for his relief. Up to this time no definite diagnosis had been made.

On June 20 patient's pains were slightly decreased; had one vomiting spell; lay rather quietly in bed under opiates.

On June 23 the patient's temperature was 103°, and his abdomen was now tympanitic. Blood cultures taken on June 22 and 23 proved negative.

On June 24 the patient was depressed and had the facial expression of one suffering; the knees were flexed; patient was evidently in a septic condition.

On June 25 blood culture was still negative.

On June 26 patient went into collapse. His abdomen was now distended with gas, and a provisional diagnosis of peritonitis was made.

On June 27 patient went into coma, and died soon after. No surgical operation was made in this particular case.

An autopsy on June 27 revealed the following:

Body is that of a well-nourished and well-developed young man. Rigor mortis is still present; lividity is present on the posterior and dependent portions of body. There are no external physical abnormalities or scars found. Upon opening the abdominal cavity free gas escapes, and the cavity is found filled with fluid pus, yellowish in color. There is about 4.5 liters of this pus. The greater omentum is necrotic, and occupies the epigastric region. The serosa of the intestines is covered with a flocculent, fibrinous exudate. The intestines are adherent to each other. The proximal portion of the appendix is inflamed, and the gland appears necrotic. Upon removing the large and small intestines and separating the adhesions we find in the epigastric region a ruptured ulcer. This ulcer extends to the duodenum, just beyond the pyloric end of the stomach. The thoracic cavity is free of fluid. Base of left lung is adherent to the diaphragm. There is an acute fibrinous pleurisy at base of right lung, which is slightly adherent to the diaphragm.

Heart weighs 290 gm. Pericardial sac contains about 20 cc. of straw-colored fluid. Right side of heart is filled with a chicken-fat clot. Foramen ovale is closed. Mitral measures 9 cm. Mitral valve is normal. Tricuspid valve measures 12 cm. It is likewise normal. Pulmonary valve measures 6.5 cm. There is a slight atheroma just above this valve. The heart muscle on section is beefy-red, firm in consistency, but shows no gross change. The coronaries are normal.

Left lung weighs 550 gm. Pleura is everywhere smooth, except at base, where it is roughened and hemorrhagic. Bronchus is injected, and covered with a serous exudate. Pulmonary vessels are normal. On section lung shows only congestion.

Right lung weighs 600 gm. Externally it is similar to the left. Bronchus of this lung is likewise injected. On section the organ shows only congestion and a moderate amount of edema.

Aorta is normal.

Bladder is normal.

Ureters are normal.

Liver weighs 2,150 gm. On section lobules are indistinct. Liver is the seat of a diffuse parenchymatous degeneration. Gall-bladder contains no stones.

Spleen weighs 250 gm. Capsule is thickened, and covered with an organized fibrin. On section organ shows sepsis. The Malpighian bodies are not as distinct as normally.

Left kidney weighs 300 gm. On section organ is the seat of an acute parenchymatous degeneration.

Left adrenal measures 3 x 4 cm. On section it is a deep black in color; consistence is firm, and it is with difficulty that one can distinguish the medullary from the cortical area.

Right kidney weighs 300 gm. On section this organ is similar to the left.

Right adrenal is the seat of a diffuse hemorrhage. Its measurements are the same as the left. On section it presents a condition perfectly similar to the left, the gland being a deep black in color; dry, and on section it is impossible to tell the difference between the cortical and medullary zones. On dissection of the left adrenal vein the lumen is blocked with a mixed white and red thrombus, completely distending its lumen, and stopping rather short in a "finger-gloved" appearance at its entry into the renal vein. A similar thrombus is found in the right adrenal vein, which completely blocks the lumen. This thrombus is firm, red and white in color, and likewise stops short at its entry into the inferior vena cava.

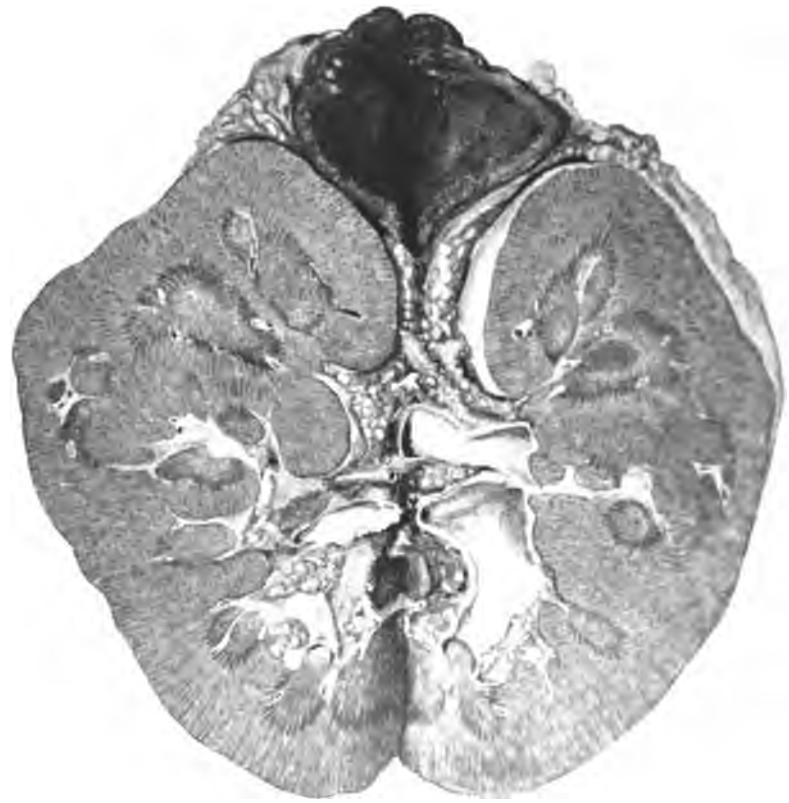

Fig. 2. Hemorrhagic Infarction of Suprarenal in a Case of Perforating Duodenal Ulcer.

Duodenum shows an ulcer about 5 cm. in diameter, which has ruptured into the abdominal cavity.

Anatomical Diagnosis: Bilateral fibrinous pleurisy at bases of lungs; congestion of lungs; septic spleen; cloudy swelling of liver; acute parenchymatous degeneration of kidneys; generalized acute purulent peritonitis, following a rupture of duodenal ulcer; bilateral infarction of adrenals.

This case is one of unusual interest and of great pathological rarity, and only a brief resumé of the condition is presented at this time. It may be well to note that all the writers on thrombosis have failed to describe a condition similar to the one which is presented here in this case. Not even Welsh in his memorable lectures on embolism and thrombosis mentions it, and a close review of the Goulstonian lectures on the suprarenal bodies by Rolleston fails to reveal any mention of this lesion.

The intensity of the infarction is similar in color and consistence to those infarctions that we find in the lung.

The intensity of the infarction is great (Fig. 2) and it may be well to repeat here what Welsh says in his lectures on embolism and thrombosis: "That if the veins are obstructed sufficiently to render the outflow nothing, or exceedingly small, and if the arteries are at the same time open, the infarction is intense."

It is exceedingly difficult to trace the origin of the thrombus in both adrenal veins. Undoubtedly its occurrence in a case of septic peritonitis following a duodenal ulcer which had perforated is a factor. Indeed, one might say that in origin it was probably of bacterial marantic thrombus.

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A SARCOMA IN A GUINEA PIG

F. C. WOOD, M.D.

(From the George Crocker Special Research Fund, Columbia University, New York.)

On May 19, 1915, the George Crocker Special Research Fund received, through the kindness of the staff of the Research Laboratories of the Department of Health of the City of New York, an adult female guinea pig, weighing 710 gm. and bearing a tumor on the right foreleg. The growth, which was pear-shaped, was

6 cm. long, 5 cm. broad at the widest portion, and 2 cm. at the narrowest; it was hard and not attached to the skin. At operation, under ether anesthesia, the tumor was found to be so intimately connected with the bone that amputation of the leg was required for its extirpation. The neoplasm weighed 60 gm. and was firm in consistency and whitish in color; it appeared to spring from the ulna.

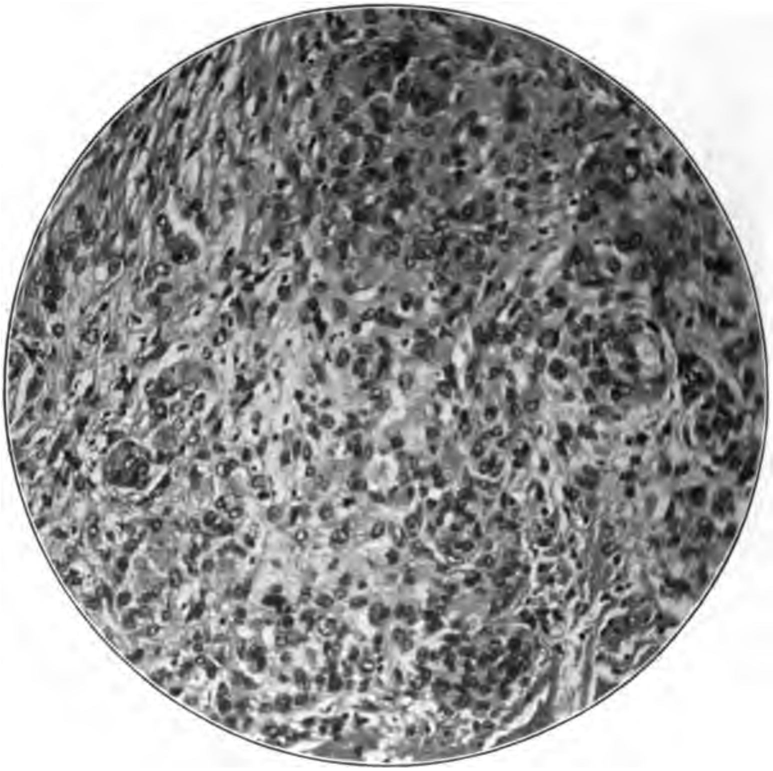

FIG. 1. Section of primary tumor showing richly cellular area. × 200.

Six grafts were inoculated into the pig herself, and the remainder of the mass was preserved for transplantation and for histological study. Unfortunately, the pig died during the following night; an autopsy showed nothing except an ovarian cyst and pregnancy. No gross metastases were discovered after a most careful search.

The tumor was transplanted into 569 young guinea pigs, weighing from 180 to 200 gm., 0.01 gm. in the right and 0.02 gm. in the left groin. Tumors began to develop in these pigs on July 7, 1915, forty-nine days after inoculation, and continued to appear up to December 15, or 210 days after inoculation. More tumors were obtained from the 0.02 gm. dose than from the smaller, though not a few developed from the 0.01 gm. grafts; in several pigs both grafts gave rise to tumors.

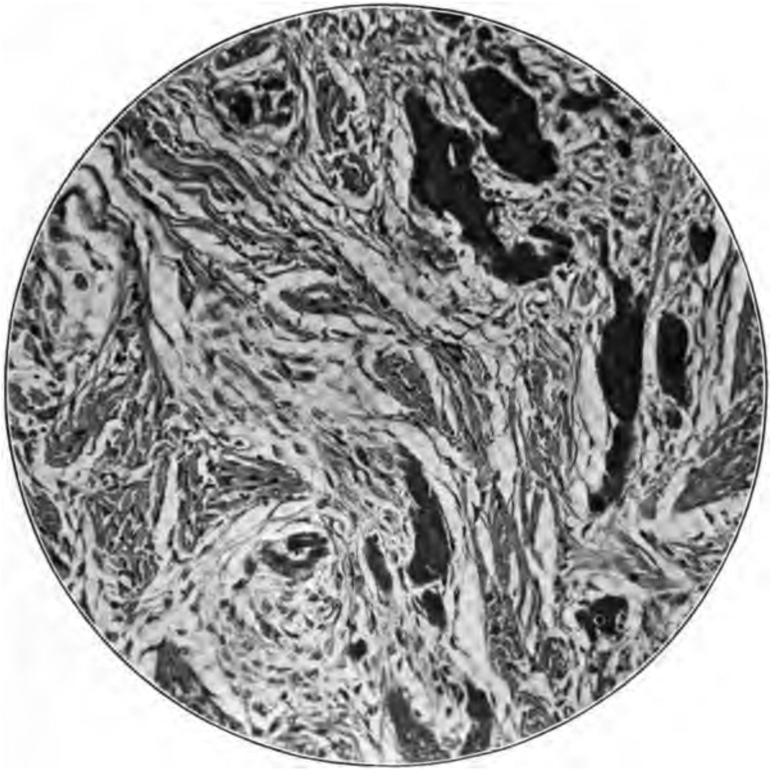

FIG. 2. Section of primary tumor showing areas of calcification. × 200.

Of the 569 pigs, 34 have developed tumors varying from the size of a small green pea to that of a hen's egg. A few of the smaller nodules have disappeared, but the great majority are still growing progressively. The percentage of "takes" might have been larger had not 374 of the pigs been lost in an epidemic. The basis for calculation is, therefore, 34 positives in a total of 195 pigs, or 17 per cent. All the growths so far examined have been replicas of the spontaneous neoplasm.

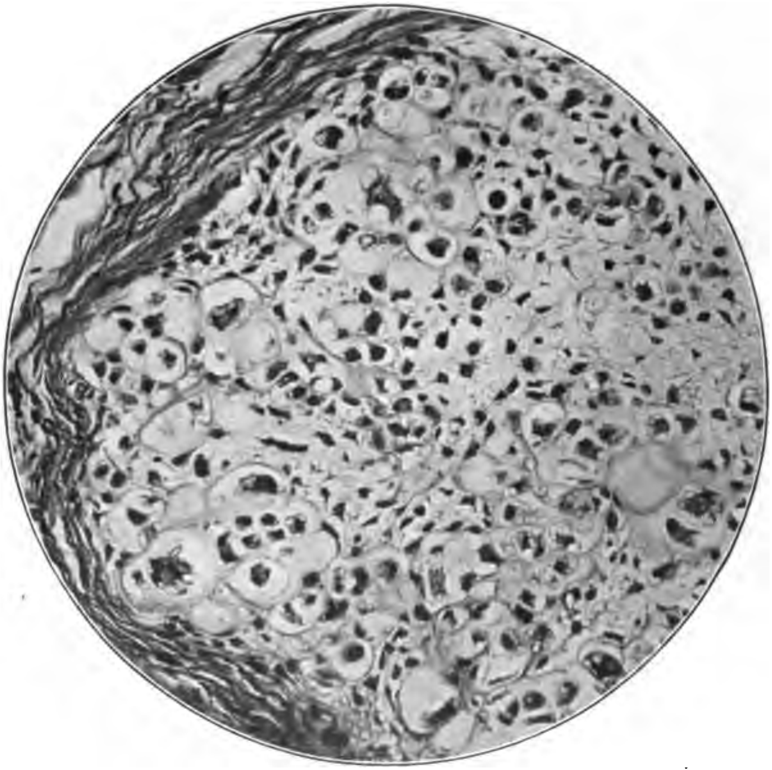

FIG. 3. Section of primary tumor showing cartilaginous area. × 200.

The second generation has not proved so adaptable. By the first transplantation, September 30, 1915, one receding tumor was obtained in 109 pigs, 58 of which have survived until now. The second, November 26, 1915, gave one progressively growing neoplasm in 138 pigs, 125 of which are still living. A third series, transplanted December 1, 1915, has so far produced no tumors in 250 pigs, 210 of which are still living. Other series, comprising several hundred animals, have been laid down too recently for tumors to appear.

On microscopical examination, the tumor was found to be a fibrosarcoma. The proportion of fibrous tissue varied in different parts of the growth (Fig. 1), the more vascular areas being more cellular. Some portions of the tumor were somewhat edematous and others were very firm. The tumor involved the periosteum and had invaded the medullary cavity of the bone. Scattered throughout the connective tissue near the bone were a number of calcified areas (Fig. 2), and there was one spot which suggested cartilage (Fig. 3), but might be entirely due to trauma. No cartilage was found in the first generation of transplanted tumors; the type of growth remained the same except that it was perhaps a little more cellular (Fig. 4) and contained more cells with ir-

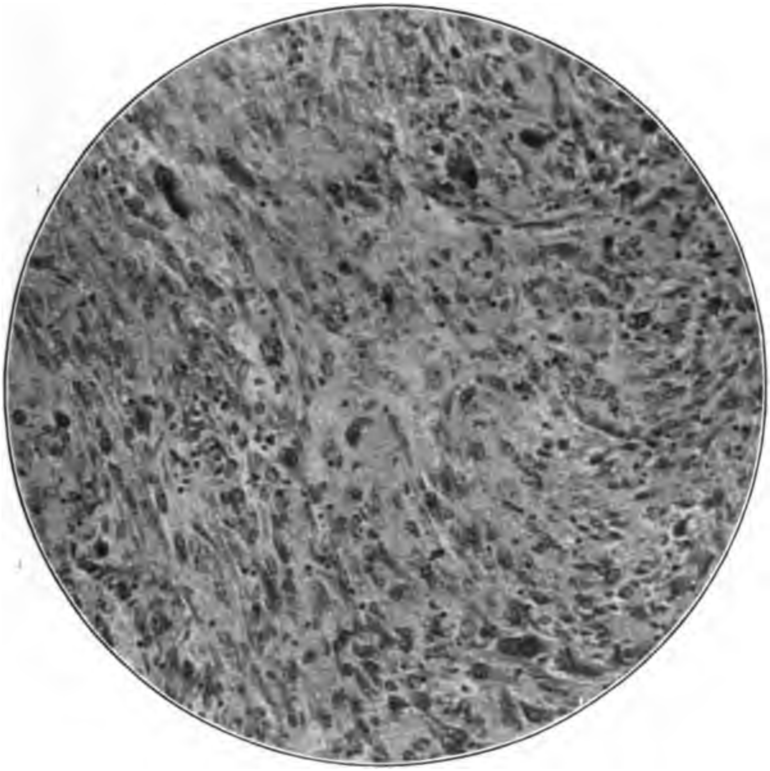

FIG. 4. Section of tumor in first generation. × 200.

regular nuclei. Mitotic figures were not infrequent both in the spontaneous growth and in the first generation of transplants.

No metastases were discovered in sections from the heart, lungs, liver, spleen, pancreas, kidneys, adrenals, and ovaries.

Only two other tumors of the guinea pig have, so far as is known, been reported; one by Murray[1] and the other by Dickson.[2] The latter could not be transplanted. Murray's tumor was a very slow growing fibrosarcoma, closely resembling the one herein described.

Discussion:

Dr. Levin: Is it possible that there is some relationship between the fact that guinea pigs are especially sensitive to the introduction of foreign proteids and the fact that the occurrence of malignant tumors in these animals is rare?

Dr. Zinsser: As a matter of fact it is difficult to produce anaphylaxis in mice, and Ritz and Sachs believe that the complement of mice contains only the mid-piece. However, it would seem to me that this could hardly be taken as support of any such bold theory applied to cancer. In fact, we know very little about anaphylaxis in mice.

Dr. Wood: Dr. Levin's anaphylactic theory does not agree with the fact that rabbits do not often have tumors, and at the same time are not very sensitive to anaphylaxis.

THE HISTOPATHOLOGY OF DISEASES OF THE APPENDIX

ELI MOSCHCOWITZ, M.D.

The purposes of this report are two: first, to attempt to clarify some current ideas concerning the pathology of appendicitis; and second, to demonstrate and establish definite criteria by which disease of this organ may be recognized. Pioneer work in this field was performed by Aschoff and since the publication of his work but little has been added to the subject. The present report is the result of the study of a large series of appendices, about 1,500 in all. The various diseases will be discussed in order, beginning with acute inflammations.

The earliest lesion in acute appendicitis is quite characteristic.

---

[1] Murray, J. A., Jour. Path. and Bacteriol., 1916, xx, 260.

[2] Dickson, E. C., *Proc. Soc. Exper. Biol. and Med.*, 1915, xiii, 26.

It begins with a loss of epithelium and the formation of an exudate which appears first at the base of each crypt of the appendix and extends from there into the lumen. At the same time, there is an infiltration of polymorphonuclear leucocytes throughout all the coats, and a localized peritonitis. Every case in this series shows a localized peritonitis, but it must be admitted that in no case was the appendix removed less than 11 hours after the onset of symptoms. As the acute process advances, there is greater and greater destruction of mucosa, and in the more advanced stages the lumen is completely filled with exudate. The infiltration of the muscular coats also becomes more intense until it is finally impossible to recognize any of the histological components of the appendix. Ultimately, necrosis supervenes, resulting in so-called gangrene. The lymphatic apparatus of the appendix has a peculiar immunity from the destructive process. The extent of the acute lesion corresponds in general to the duration of the illness.

The following conclusions may be drawn from the study of the pathological anatomy of acute appendicitis: *first,* there is every ground for presuming that the infection is enterogenous rather than hematogenous in origin; *second,* the lesion is not a catarrhal inflammation in the usual meaning of the term; *third,* the lesion is the result of bacterial infection and begins in the crypts because stagnation is more apt to occur here; *fourth,* the pathological lesion corresponds fairly accurately to the duration of the illness; *fifth,* the lesion is a membranous and suppurative inflammation; a membranous inflammation is in fact the usual type in all acute infections of the mucous membranes; *sixth,* the best term by which to designate the lesion is "acute appendicitis." All other terms, such as gangrenous, ulcerative, catarrhal, etc., should be discarded.

In studying the pathological anatomy of acute perforation of the appendix it is found that perforation usually occurs only in the later stages of the malady. Rupture is apparently due to necrosis with increased tension of the intra-appendicular contents. It is unlikely that thrombosis of the mesentery is an important factor in causing perforation.

The majority of empyemata of the appendix seem to be due rather to the accumulation of purulent material in a previously dilated portion of the appendix, extending from an adjacent acute lesion of the organ, rather than to a primary acute inflammation at the site of the empyema with secondary dilatation. The second chain of events may occur but it is exceptional.

In healing appendices one finds in some specimens an ulcer which shows beginning ingrowth of epithelium from the adjacent normal mucosa. In other specimens there is complete destruction of the mucosa with replacement by granulation tissue. There are concomitant healing processes in the other coats of the appendix. The course of events is the following: the exudate or membrane breaks down, becomes necrotic and leaves an ulcer covered with granulation tissue. If the mucosa has been completely destroyed there is complete obliteration of the lumen. If the mucosa has been only partially destroyed a stricture is formed. At the same time, the formation of new connective tissue in the muscular coats leads to two changes—deformity and separation of the muscular fibers. The peritoneal coat may undergo thickening and adhesions may be formed.

The healed or chronically diseased appendices studied were of two types, those showing stricture and those showing obliteration. In addition these appendices showed the following lesions which are characteristic of chronic appendicitis: *first,* absence of mucosal crypts; *second,* widening of the submucous connective tissue; *third,* diminution, or even complete absence of the lymphoid tissue; *fourth,* infiltration and deformity of the muscular coats; and *fifth,* thickening, increased density and deformity of the peritoneal coat. All of these conditions result from primary acute lesions. It is conceivable that if the acute process were arrested early there might be a complete restitution to the normal. Such an occurrence did not take place, however, in any of the cases studied, and the conclusion seems warranted that an appendix which is anatomically normal has never been the seat of an acute attack.

The lesion of chronic appendicitis is in every sense an end product and not a progressive and continuous inflammatory process. This study has shown no lesion which could be classed as a "chronic catarrhal appendicitis." The chronic condition is always the sequel of an acute attack, even though clinically there be no history of such an attack.

A number of lesions were observed which simulate appendicitis:

(*a*) Submucous hæmorrhages in the appendix. These are due to trauma at operation.

(*b*) Coproliths. These are probably not a direct cause of an acute attack. They only influence the process by interfering with the drainage of the organ.

(*c*) Lymphoid hyperplasia of the appendix. This lesion is almost always associated with a status lymphaticus.

(*d*) Oxyuris in the appendix. This is an exceedingly common finding. In no case could an invasion of the coats of the appendix by the worm be observed. As a rule the presence of oxyuris gave no symptoms.

(*e*) Trychocephalus dispar.

(*f*) Decidua of the appendix. The decidual cells are found in the subperitoneal connective tissue. At least two dozen appendices, removed in the course of operations for ectopic pregnancy, were examined, but this lesion was observed only once. It should be regarded as merely another evidence of the widespread decidual reaction in the pelvic connective tissue that occurs normally in pregnancy.

(*g*) Intra-appendicular mucocele. The appendix shows evidence of a chronic inflammation. The lesion is characterized by a complete conversion of the appendicular epithelium into the mucous or goblet type. These appendices sometimes rupture, leading to the strange phenomenon known as muco-peritoneum.

(*h*) Extra-appendicular mucocele. This is a small connective tissue vesicle on the peritoneal surface filled with mucus. The genesis of mucoceles is not clear but it may be related to a previous perforation of the appendix.

(*i*) Diverticulum of the appendix. Two cases were observed.

(*j*) Carcinoma of the appendix. Three types are found, scirrhus, medullary and adeno-carcinoma. The lesions may be found in association with acute appendicitis. Carcinoma of the appendix is comparatively non-malignant both from the clinical and the pathological point of view.

CONCLUSIONS

I. The pathological lesion of acute appendicitis represents a suppurative process from the very beginning. The earliest lesion is as characteristic as the primary lesion of syphilis, and all the subsequent stages of the disease within the organ are directly traceable to the spread and development of this lesion. There is no pathological evidence whatever that an acute catarrhal inflammation of the appendix occurs.

II. The changes grouped under the name chronic appendicitis —stricture, obliteration, etc.—are the result of the healing of the acute lesion. According to this interpretation, chronic appendicitis is not a continuous and progressive inflammation, but an end product. There is no pathological evidence that involution of the appendix or chronic catarrhal inflammation of the appendix occur.

III. The only justifiable classification of inflammations of the appendix, therefore, is the following:

(*a*) Acute appendicitis.
(*b*) Healing or subacute appendicitis.
(*c*) Healed or chronic appendicitis.

IV. An acute localized peritonitis with the formation of fibrin and limited to the site of the lesion is always present in acute appendicitis as early as 12 hours after the onset, and perhaps earlier, consequently the absence of a localized peritonitis on gross inspection of suspected cases is in itself indicative of absence of acute appendicitis.

V. In addition to obliteration and stricture, attention is called to two new pathognomonic signs of chronic appendicitis, which are constant and easily recognized, namely, absence of mucosal crypts and marked widening of the submucous connective tissue zone.

The latter change is especially easy to detect upon cross section of the organ, and is valuable as the simplest indication of the presence or absence of a chronic appendicitis.

VI. Cross-sections of the appendix at various levels are far preferable to longitudinal sections for the study of pathological changes.

Discussion:

Dr. Larkin: It seems to me that Dr. Moschcowitz has given us a great deal of cause for reflection in presenting Aschoff's idea of appendicitis. This, to my mind, is not in accord with many surgeons' ideas as to what appendicitis is or is not. To those of us who have been examining appendices for years the pictures presented are familiar. Dr. Moschcowitz has put a great deal of study into this paper, particularly into the pathological anatomy, and I think he is to be congratulated. There is one picture which he has presented this evening which has an important medico-legal bearing. I refer to the section of an appendix in which decidual cells are seen beneath the peritoneum. This observation was first made by Pick, who discovered beneath the peritoneum of the uterus in Douglas' cul-de-sac several small nodules, which, upon microscopic examination, proved to be decidual cells. This was a novel find at the time, but it is now being gradually recognized as of great importance in its connection with pregnancy. It may be of great medico-legal interest, especially where the uteri are cleaned out so completely by an abortionist as to leave no vestige of placental or decidual remains. In examining a large number of uteri after abortion and pregnancy, I have been surprised at the frequency of decidual remains after careful microscopic examination, which were identical with the picture Dr. Moschcowitz presented this evening. Perhaps all of us cannot agree with his classification, several points of which he mentions as having been accepted by most pathologists. His classification, in my opinion, seems rather radical.

Dr. Pappenheimer: Because after twelve hours lesions are found in the mucosa, it does not, I believe, follow that the infection was originally enterogenic rather than through the blood stream. Dr. Moschcowitz has pointed out the coexistence of a local peritonitis at this time, indicating that the distribution of the organisms through the wall of the appendix is quite widespread. In the case of early tuberculosis of the Fallopian tubes, in which the infection is unquestionably hematogenous, the lesions are first seen in the mucosa and rapidly lead to a destruction of the overlying epithelium. It would seem to me, therefore, that Aschoff's views, which Dr. Moschcowitz seems to have adopted, while probably correct for most cases, can hardly be considered as absolutely substantiated. I should like to ask also whether Dr. Moschcowitz believes that senile involutionary changes in themselves may lead to obliteration of the appendix?

Dr. Wood: I recently examined an appendix which had been removed from a patient suffering from an acute attack of appendicitis with fever and a leucocyte count of 26,000. The whole lumen of the organ was changed into fibrous tissue and yet there was an acute suppurative process in the subserosa. The patient denied any previous attack. It is rather difficult to put such appendices into an ordinary classification.

Dr. Lambert: I was rather disappointed to hear Dr. Moschcowitz say that he regarded the so-called carcinomata of the appendix as true cancers. It seems to me that the term carcinoma should not be applied to any but malignant epitheliomatous growths and that the only safe criterion of malignancy is the behavior of the cells which compose the tumor. The mere fact that cells are arranged in solid nests does not afford a sufficient basis for calling them carcinoma cells. That the majority of these tumors of which Dr. Moschcowitz has reported several typical examples show little tendency to invasive growth and do not metastasize is well known. The cells, furthermore, as he has pointed out, do not resemble those seen in most malignant tumors. They are small and usually show deeply staining nuclei. One rarely finds mitotic figures among them. It appears to me, therefore, that we are not warranted in applying the term carcinoma to such growths.

Dr. Ewing: I agree with Dr. Larkin that the reader of the paper deserves much credit for bringing out so much of interest in this important subject. I would like to ask if the designation of chronic catarrhal appendicitis does not apply to mucocele. That seems to me legitimate. I was not aware that they filled the abdominal cavity with mucus. I thought that was from a tumor of the peritoneum. Most of us would agree with the general statements made, but perhaps there is too great finality about some of the conclusions. I do not think you can always say of a lesion that it does this or that invariably. That is a hazardous view to take. Dr. Delafield recognized several types of appendicitis, the catarrhal, purulent, exudative, necrotic and gangrenous. I confess I have been teaching that classification several years to students. I have seen sections which reflect these types. I don't always find peritonitis on the surface. In very acute stages peritonitis occurs. I have seen what I would regard as early gangrenous appendicitis. This, I think, is determined by extensive thrombosis of the vessels. Generally speaking, Dr. Moschcowitz is correct as regards the great majority of surgical specimens. One finds catarrhal enteritis and catarrhal colitis, so also appendicitis. Surgeons don't see these but I think they occur.

I cannot refrain from discussing Dr. Lambert's remarks on the cancer question. It is known that these tumors differ from all other forms of carcinomata. In different organs cancers do not act alike. There are some appendix carcinomata which do metastasize and do infiltrate, but not excessively. They lack the complete clinical picture of cancer. So do rodent ulcers. If the appendix types had ten or fifteen years to develop they might express their capacity for malignancy.

Dr. Levin: I want to express my appreciation of the wonderful erudition of Dr. Moschcowitz's paper. It is unusual in these days to find a man willing to study a subject for six years until he gets ready to say something. One word in regard to cancer of the appendix—during my practice I can remember two clinical cases where the condition began as an acute appendicitis with all the clinical symptoms. The patients refused operation. Ultimately operation showed a carcinoma of the cæcum. I believe that carcinoma of the appendix is so frequently found to be circumscribed because the clinical symptoms appear sooner than in almost any other malignant tumor.

Dr. Moschcowitz: In answer to Dr. Pappenheimer's remarks, the reason I believe appendicitis is always enterogenic in origin is because the lesion always begins in the mucosa and is associated with a distinct loss of epithelium. Moreover, I have never seen a focal infection in the walls of the appendix unassociated with a mucosal lesion.

In regard to Dr. Wood's case the question of the genesis of certain obliterated appendices has puzzled me greatly. In the vast majority of instances a previous history of one or more attacks of appendicitis can be elicited, especially after a careful cross-examination. In a few no history of anything resembling a previous attack is obtainable. However, from a pathological point of view, I can not conceive that extensive obliteration of the appendix could occur without previous destruction of the mucosa.

In answer to Dr. Ewing I agree and have so stated in this paper, that mucoceles are consequent to chronic inflammations of the appendix. I would assure Dr. Ewing that extensive collections of mucus can collect in the peritoneal cavity from simple rupture of mucoceles of the appendix or old pus tubes. I have myself seen such an instance. It seems impossible to obtain a precise difinition of the term "catarrhal inflammation." As I understand it—and the majority of pathologists whom I have questioned agree—a "catarrhal inflammation" of a mucous membrane is characterized by congestion and exudation of mucus.

Dr. Ewing: A catarrhal inflammation is marked by an exudate of serum, pus, or mucus, thrown off a surface by desquamation of the lining cells.

Dr. Moschcowitz: Most pathologists do not agree with the view of Dr. Ewing that a catarrhal inflammation is associated with pus. They class such inflammations as suppurative.

Dr. Ewing: Volkmann accepts that definition.

Dr. Moschcowitz: Others do not, and especially Aschoff. Dr. Ewing criticised some of the statements in the paper as perhaps too categorical. I have only made such statements categorical, as I believe my observations demanded. In regard, for instance, to the question of localized peritonitis in acute appendicitis, my observations upon many hundreds of appendices removed during an acute attack entitle me to say definitely that a localized peritonitis is always present in every case of acute appendicitis. I have never seen an exception to this rule.

In regard to thrombosis of the mesentery, I am unwilling to state how often it occurs, because I have been unable to determine whether the thrombosis was ante- or post-operative.

STUDIES ON TREPONEMA PALLIDUM AND SYPHILIS

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(*a*) FLUCTUATIONS IN VIRULENCE OF INDIVIDUAL STRAINS AND COMPARATIVE VIRULENCE OF DIFFERENT STRAINS OF TREPONEMA PALLIDUM IN RABBITS

Incident to other laboratory experiments on treponemata several strains have been kept going in rabbits and the fluctuations in virulence, incubation time, character of lesions, etc., observed.

Five strains have been kept alive through from ten to more than twenty generations in rabbits. Four of these were obtained from chancres and mucous patches; the fifth is a strain with which Dr. Nichols worked and which he very kindly sent to this laboratory. It has appeared that fluctuations in virulence, differences in incubation time and differences in the character of the lesions obtained have depended, not on the special virulence of any particular strain, nor upon the number of passages through rabbits, but upon various other factors:

1. The character of the rabbit lesion from which the inoculation was made; young, fresh, diffuse lesions giving a higher degree of virulence and a shorter incubation time than older, more indurated lesions.

2. The size of the testicles of the rabbits which were inoculated; the higher percentage of takes with a shorter incubation period being obtained in rabbits with well-developed testicles.

3. The technique of the inoculation, a thorough maceration of the tissue to be inoculated and a thorough injection of the material all through the testicle tending to decrease the incubation time and increase the virulence.

4. Absence of delay between removal of lesion and inoculation into fresh rabbits.

5. The character of the lesion has seemed to depend considerably on whether it developed in the testicle or in the firmer tissues of the sac.

Upon these various factors have seemed to depend all changes in virulence, incubation time, or character of the lesion. All kinds of lesions and variations in incubation time have occurred in each one of the five strains and frequently in the same generation, while no increase in virulence has been observed after repeated passage through rabbits. Noguchi has described three types of treponemata which differ in thickness and in their virulence for rabbits. We have been unable to identify these in any particular kind of lesion or any particular strain. In fact, we have seen what appear to be thick and thin types in the same preparation.

In regard to the generalization of the disease in rabbits, we have noticed three cases of keratitis, each one occurring in a different strain. Aside from these and a few minor lesions, such as involvement of the prepuce and in one case demonstration of the treponemata in the peritoneal cavity, we have noticed no tendency to generalization in any of the strains.

(*b*) GROUP AGGLUTINATIONS OF TREPONEMATA IN PALLIDUM ANTISERUM

A year ago we reported the observation of agglutinative substances for *Treponema pallidum* from cultures, in the serum of rabbits immunized to these cultures. Following this observation we wished to determine the specificity of the reaction, whether group reactions might be expected in the blood of individuals infected with species of treponemata other than pallida, and also whether the reaction might be used for the identification of cultures. In carrying out these tests we have used the serum of immunized rabbits and of an immunized sheep. The material for injection has been obtained by taking cultures in sheep serum broth to which kidney tissue had been added, at their height of growth (from 3 to 6 weeks) centrifuging the treponemata from the growth at high speed, resuspending the sediment in salt solution and killing the organisms by heating at 56° for half an hour. In case precipitate was present in the suspensions, this was removed by short centrifugation at low speed. The sheep at the time these experiments were made had received 22 injections over a period of eight months of from 10 to 25 c.c. each of concentrated *pallidum* suspension. One of the rabbits (No. 624) received 30 injections over a period of 12 months of about 1 c.c. each. Another rabbit which had received only 9 injections over a period of two months showed nearly as powerful agglutination.

In carrying out the tests, suspensions of treponemata were used prepared like those used in immunization, except that the cultures were grown in ascitic broth instead of in sheep serum broth to avoid confusion from protein anti-protein reactions. The suspensions were freed as nearly as possible from precipitate and were used unheated. In our first experiments the macroscopic reaction was so striking that we depended entirely on reading with the naked eye. We soon found, however, that occasionally false results were obtained in this way. Consequently in our recent experiments, including all those reported here, the macroscopic readings have in every case been checked by examination of the sediment in the dark field. The tubes have been incubated for one hour at 37° and allowed to stand over night in the icebox before making readings.

Our preliminary experiments showed that our own strain of *pallidum*, "A," and the strains sent to us by Dr. Noguchi reacted quite similarly in sera obtained by immunizing to strain "A." On testing this serum with other species of treponemata, for cultures of which we are also indebted to Dr. Noguchi, we found there were very strong group reactions. The following is a type of such experiments:

GROUP AGGLUTINATIONS

Serum of Rabbit 624 Immunized with Treponema Pallidum (Strain A)

Treponema pallidum, Strain " A," agglutinated in dilution of	1 : 2000.
Treponema pallidum, Noguchi Strain 1, agglutinated in dilution of	1 : 400.
Treponema pallidum, Noguchi Strain 2, agglutinated in dilution of	1 : 1000.
Treponema calligyrum, agglutinated in dilution of	1 : 4000.
Treponema refringens, agglutinated in dilution of	1 : 200.
Treponema microdentium, agglutinated in dilution of	1 : 1000.
Treponema mucosum, agglutinated spontaneously in the salt solution control.	

The spontaneous agglutination of *Treponema mucosum* has made it impossible to obtain satisfactory readings with this organism.

We do not think that too much stress should be laid on the various strengths of reaction with the three strains of *pallidum*, as different suspensions of the same strain tested against the same serum will sometimes show similar differences.

For further information as to the immunological relationship to these organisms, we resorted to absorption tests. The strength of agglutination of all of these organisms in the serum used was first determined as in the following table, and then the concentrated serum mixed with a very thick suspension of the organisms with which it was to be absorbed, usually one part of undiluted serum with nine parts of suspension. After standing for some time the mixture was centrifuged and the supernatant fluid was pipetted off and treated with fresh suspension, and the process was repeated until the supernatant fluid no longer agglutinated the particular organism being used. When the absorption was complete, the serum, which by this time had been diluted from 1 to 25, to 1 to 50 was tested against several different species of treponemata. A type of such experiment is shown in the table on page 18.

As will be seen by the table, the strain of *pallidum* used and also the *Treponema calligyrum* removed all the agglutinins from the serum, whereas the *Treponema refringens* and *microdentium* removed only their respective minor agglutinins. The tonsil organism removed none. The results of other experiments have been quite similar except as to *Treponema refringens*, some suspensions of which have agglutinated very strongly in anti-*pallidum* serum and have absorbed a considerable amount of the major agglutinins. As these results were obtained only occasionally, we are inclined to attribute them to non-specific adsorption by protein particles in the suspension.

The treponema called tonsil organism in these experiments is from a culture which we isolated from an ulcerated tonsil, which is morphologically very similar to the *Treponema micro-*

dentium, but which reacts differently in the agglutinin absorption test.

ABSORPTION OF AGGLUTININS

Preliminary Titration of Serum of Rabbit 323 Immunized with Treponema Pallidum (Strain A)

Suspension of	1 : 10	1 : 50	1 : 100	1 : 200	1 : 500	1 : 1000	1 : 2000	1 : 5000	Salt
Pallidum A	+	+	+	+	+	+	+	o	o
Calligyrum	+	+	+	+	+	+	+	o	o
Refringens	+	+	+	+	o	o	o	o	o
Mucosum	o	o	o	o	o	o	o	o	o
Microdentium	+	+	+	+	+	o	o	o	o
Tonsil organism	+	+	+	+	o	o	o	o	o

Agglutination in Serum of Rabbit 323 (1:50) after Absorption

Serum (1 : 50) Absorbed with:	Tested Against Suspension of:			
	Pallidum	Calligyrum	Refringens	Microdentium
Pallidum A	o	o	o	o
Calligyrum	o	o	o	o
Refringens	+	+	o	+
Mucosum	+	+		
Microdentium	+	+	+	o
Tonsil organism	+	+	+	+
Serum untreated	+	+	+	+
Salt solution	o	o	o	o

We conclude from these observations that the different species of treponemata which we have tested are very closely related, and that the *calligyrum* is indistinguishable from the *pallidum* in these reactions.

We had some hope also that this reaction of agglutination might be of value in diagnosis, and tested first the serum of syphilitic rabbits to see whether it contains more agglutinative substance for the culture pallidum than does normal serum. Normal rabbit serum agglutinates *pallidum* frequently in dilutions of 1:10, and occasionally shows feeble agglutination in 1:20 and 1:25. Many of our syphilitic rabbits show no stronger agglutination than this, but some show a slight but distinct increase in agglutinative power. The protocol given below is typical of the results obtained in such tests.

AGGLUTINATION OF TREPONEMA PALLIDUM IN RABBIT SERA

Normal Rabbits

	1:10	1:25	1:50	1:100	1:1000
1.	+	0	0	0	
2.	+++	+	0	0	
3.	0	0	0	0	
4.	+++	0	0	0	
5.	+++	+	0	0	

Syphilitic Rabbits

Time since first appearance of syphilitic lesion					
1. 11 days	+++	++	0	0	
2 27 days	+++	0	0	0	
3. 19 days	+++	+	0	0	
4. 2 weeks	++	++	+	0	
5. 11 months	+++	+	0	0	
6. 11 months	++	++	+	0	

Rabbit Immunized with Culture Pallida

1.	+++	+++	+++	+++	+++

The syphilitic rabbit, No. 5, was one that had been twice inoculated; the first lesion developed 11 months ago; the rabbit recovered, was again inoculated, and again developed the disease. The sixth rabbit was one which had a testicular lesion 11 months ago and recently developed keratitis.

The reactions in these animals are slightly stronger than the normal, but are insignificant as compared with the powerful agglutination observed in the rabbit immunized to culture treponemata.

(*c*) TREPONEMACIDAL ANTIBODIES AGAINST TREPONEMA PALLIDUM

After observing the agglutinating effect of the serum of rabbits immunized to cultures of *Treponema pallidum*, we decided to test this serum for treponemacidal activity. The technique of such experiments proved not to be difficult provided that the proper amount of living treponemata was used for the test. On account of the absence of distinct colony formation, it is impossible to test for any partial killing of the treponemata as is done in the plating method for detecting bactericidal antibodies, and in their case only complete killing or failure to kill completely can be detected. If too small an amount of inoculum is used, successful plants are not regularly obtained, and if too large an amount, the immune serum fails to kill completely.

In preliminary experiments we found that suspensions made up so that 10 to 15 treponemata per darkfield were visible were suitable for the work if 0.1 c.c. of such a suspension was used to inoculate the tubes. The sera tested were those of rabbits and of a sheep immunized against treponema cultures as described in the previous paper, and the sera of normal animals of the same species were used for control. The reactions were set up by distributing the amounts of normal and immune serum to be tested in the high, narrow test tubes used for cultivation of the organism, the total volume in the tube being brought up in each case to 1 c.c. The tubes were then inoculated with an equal amount of broth culture of treponemata and incubated in the waterbath for two hours at 37° C. At the end of this time a small piece of sterile rabbit kidney and a deep layer of ascitic agar covered with liquid petrolatum were added to each tube. The tubes were then incubated for two weeks or until the control showed profuse growth, and then examined microscopically for growth. The tests were set up in duplicate to avoid confusion from a failure of growth due to accidental causes.

The results showed that the normal rabbit serum was treponemacidal when as much as 1.0 c.c. was used, but seldom showed any treponemacidal effect if smaller amounts were employed, whereas immune serum was treponemacidal in amounts as small as 0.01 c.c. and quite possibly in smaller amounts than this, as we did not attempt a quantitative examination of the treponemacidal effect in all cases. The treponemacidal activity of the immune serum was destroyed by heating to 56° C. for half an hour, and such inactivated serum could be reactivated by the addition of 0.05 c.c. normal serum, an amount which in itself showed no treponemacidal activity. The analogy to the bactericidal antibodies was in this respect complete.

(*d*) AGGLUTINATION OF TREPONEMA PALLIDUM IN NORMAL AND SYPHILITIC HUMAN SERA

The observation of powerful agglutinins in the sera of rabbits immunized to cultures of *Treponema pallidum* led to the hope that similar agglutinins might be demonstrated in the sera of syphilitic patients. It has been recently claimed by Kissmeyer[1] that such agglutinins are found only in syphilitics and that the reaction is of great value in diagnosis, occurring in about 50 per cent. of the cases which give a Wassermann and in some that do not. Our observations on normal human sera showed that such sera frequently agglutinated *pallidum* when mixed undiluted with an equal part of the *pallidum* suspension (dilution of 1–2) and the first experiments were consequently carried out with dilutions of from 1–5 to 1–100.

A preliminary series of tests was made in our laboratory by Miss E. W. Davis. In these tests the diluted serum was mixed with equal parts of a concentrated *pallidum* suspension in narrow agglutination tubes, which were incubated for 1 hour at 37° C., allowed to stand overnight in the ice-box, and the readings then made with the naked eye. We selected for the positive cases in this series tertiary syphilitics with a positive Wassermann reaction, as it was in such cases we felt most hopeful of observing agglutination. The results of these tests (Table I) show a more powerful agglutination in some of the syphilitic sera than in the normal controls.

TABLE I

Macroscopic Agglutinations

	No Agglutination Occurred in	Agglutination Occurred in Serum 1 : 5 in	Agglutination Occurred in Serum 1 : 10 in	Agglutination Occurred in Serum 1 : 20 in
Tertiary syphilis, W. R. +, 23 cases	13 cases	2 cases	5 cases	3 cases
Nonsyphilitic diseases, W. R. 0, 5 cases	5 "	0 "	0 "	0 "
Normal individuals, W. R. 0, 4 cases	2 "	1 case	1 case	0 "

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[1] *Deutsch. med. Woch.*, 1915, xli, 306.

In observing such reactions microscopically, it was found that the macroscopic readings were occasionally deceptive—that with a treponema suspension, quite free from particles of media, a definite agglutination might be invisible to the naked eye though quite striking under the dark field. On the other hand, it was occasionally found that a flocculation occurred in the tubes in which no agglutination of the treponemata could be observed microscopically. Consequently, in further experiments, all readings were made from tubes set up and incubated as described above, by making a dark field preparation from each tube after disturbing the sediment by drawing it in and out of a capillary pipette. In such preparations, we often met with clumps of a half dozen organisms in the salt controls or in the negative sera, and consequently decided to disregard such slight clumping and consider as positive only specimens which showed large clumps (10 or more organisms) throughout the preparation. Only rarely did we observe the formation of large clumps of innumerable treponemata with complete disappearance of free organisms, an appearance seen in all of our immune sera recorded as ++++. The results of our series of tests carried out in this way are given in Table II. As the work progressed, it was found that so many

TABLE II

Microscopic Agglutinations

	No Agglutination Occurred in	Agglutination Occurred in Serum 1 : 2 in	Agglutination Occurred in Serum 1 : 10 in	Agglutination Occurred in Serum 1 : 20 in	Agglutination Occurred in Serum 1 : 50 in	Agglutination Occurred in Serum 1 : 100 in
Primary syphilis, W. R. +, 6 cases.	3 cases	1 case	2 cases	0 cases	0 cases	0 cases
Secondary syphilis, W. R. +, 18 cases	8 "	4 cases	3 "	1 case	1 case	1 case
Tertiary syphilis, W. R. +, 64 cases	27 "	7 "	23 "	3 cases	2 cases	2 cases
Tertiary syphilis, W. R. 0, 2 cases. .	1 case	0 "	0 "	1 case	0 "	0 "
Congenital syphilis, W. R. +, 4 cases.	0 cases	1 case	3 "	0 cases	0 "	0 "
Nonsyphilitic diseases, W. R. 0, 37 cases.	22 "	4 cases	7 "	1 case	2 "	1 case
Normal individuals, 40 cases.	35 "	4 "	1 case	0 cases	0 cases	0 cases

nonsyphilitic sera gave agglutination in the 1 :2 dilution that observations on sera in this concentration were abandoned after about one half the tests had been done. Consequently, it is probable that a number of the sera recorded as quite negative would have been positive in a 1 :2 dilution if tested.

As mentioned above, we decided to disregard the positive agglutination found when undiluted normal serum was mixed with the *pallidum* suspension (1 : 2) on account of the relative frequency with which such agglutination occurred in the sera of normal individuals. Summarizing Table II, we found that agglutination in dilutions of 1 :10 or above was obtained in:

Primary syphilis	in 2 out of 6 cases,	or 33 per cent.
Secondary syphilis	in 6 out of 18 cases,	or 33 per cent.
Tertiary syphilis	in 31 out of 66 cases,	or 47 per cent.
Non-syphilitic diseases	in 11 out of 37 cases,	or 30 per cent.
Normal persons	in 1 out of 40 cases,	or 2 per cent.

As compared with the sera of normal individuals, only one of which agglutinated in the dilutions regarded as significant, positive reactions were frequently observed in the sera of syphilitics. When, however, we compared syphilitic sera with sera of patients suffering from various other diseases and giving negative Wassermann reactions, we found such sera reacted almost as frequently as did the syphilitic. Occasionally also we found very powerful agglutination in cases in which there was absolutely no evidence of syphilis. These findings we are unable to explain. The possibility that the patients had an infection with some other treponema—for example, a throat infection—must be borne in mind. The strong group reactions which we have observed among different species of treponemata would make this seem probable.

The tests in syphilitic sera lead us to believe that, in some cases, especially tertiary cases, there is a small amount of antibody to the treponema developed in the patient's serum. The appearance of this serum immunity is, however, so inconstant and so slight in degree that it seems unlikely that it should have a marked influence on the course of the disease. It certainly indicates that the agglutination reaction is unlikely to be of any value in diagnosis.

(*e*) DIFFERENCES IN SERUM SUSCEPTIBILITY BETWEEN VIRULENT AND CULTURE PALLIDA

Very marked differences were observed between the behavior of virulent treponemata obtained directly from lesions in rabbits and that of treponemata which had been cultivated for a number of generations on artificial media and had completely lost their virulence for animals.

Agglutination experiments showed that the serum of rabbits immunized to cultures, which had a very powerful effect on the avirulent treponemata, agglutinated the virulent organisms very slightly or not at all. Treatment of the virulent organisms by the method of Porges did not render them susceptible to the immune serum. Treatment of virulent suspensions with immune serum before they were injected into animals showed no protective action on the part of the immune serum, although the serum showed in test-tube powerful treponemacidal action against the avirulent organisms. This is more or less in keeping with the fact that infection with the treponemata excites little antibody formation. It is possible that a more definite effect on virulent organisms would be obtained by more powerful immune serum.

(*f*) SPECIFIC COMPLEMENT-FIXATION WITH TREPONEMA PALLIDUM

The writers have utilized a method for the cultivation of *Treponema pallidum* in large quantities for the production of antigen for complement-fixation. For the production of the antigen, slanted egg medium, as for Dorset's egg, is made up in high tubes, covered with ascitic broth, and sealed with paraffine oil. No tissue is needed and the egg can be thoroughly sterilized in the autoclave. The culture treponema grows actively in these tubes so that plentiful growth can be obtained in periods as short as ten days. This enables one to work with young cultures. The material is centrifugalized, ground up with salt, and taken up in distilled water to isotonicity. The writers have paralleled the Wassermann reactions in 159 cases at the time of making this report, with entire identity in 138. There were

21 discrepancies, in a considerable number of which the treponema fixation indicated syphilis which the Wassermann missed. On the other hand, the writers do not regard their reactions, at present, as specific. Experiments indicate that similar antigens can be produced from egg cultures of colon and typhoid bacilli and that a certain amount of the fixation is due to the lipoidal constituents of the microorganism and derived in part from the egg used for cultivation. However, the apparently greater delicacy of this reaction may mean that there is a certain specific element superadded. It may mean, however, that the reaction is more delicate only because a bacterial antigen produced in this way and very delicately titrated (and it is necessary in working with these antigens to approach more closely to the anticomplementary dose) represents simply another way of doing the Wassermann. This, the writers think, is quite probably the case in the many fixations they obtained with the treponema antigen in early syphilis—cases in which their other work does not indicate the existence of antibodies in any great amount.

Further work is being done both in the direction of distinguishing the differences between the treponema antigen and similarly produced antigens from other bacteria, and fixations are being done with the protein and lipoid fractions of the treponema antigen.

Discussion:

DR. PARK: If I understood rightly, it was stated that cultures that had been long grown gave better antibodies than fresh cultures. We have felt that we got better antibodies by injecting our horses with recently isolated cultures. The results reported would throw doubt on this belief.

DR. WOOD: The question of the organism being perfectly adapted to the body so that it does not produce antibodies is a very favorite one with cancer research workers who think that the cancer cell is so perfectly adapted to its environment that the body forms no defence.

DR. PARK: I wonder whether the same amount of killed recently obtained organisms has been compared with the killed cultured *pallida.*

DR. OTTENBERG: I have had an opportunity to watch this work, and to compare the complement fixations with spirochæte antigens in syphilis in parallel tests with the Wassermann reaction, and I cannot help thinking that there must be a specific element in this complement fixation, something more than the physical effect of the antigen, for the reason that the discrepancies come out in a very interesting way. While it was astonishing how regularly the two reactions agreed, when they disagreed usually the positive reaction with the spirochæte antigen was obtained in a case of tertiary syphilis in which the Wassermann reaction had been positive and had become negative under treatment. The cases in which the Wassermann was positive and the spirochæte test negative were nearly all cases of very early syphilis. If the fixation were due to a physical or chemical reaction with substances other than antibodies in the blood, one would expect it to run parallel with the Wassermann reaction. In a very few cases with no suspicion of syphilis a positive spirochæte fixation was obtained. This may have been a group reaction, such as could perhaps be given if the patient had antibodies for Vincent's spirillum or various spirochætes found in the mouth. The finding of a strong positive Wassermann, with a negative spirochæte reaction in early cases of syphilis, is what one might expect if the spirochæte reaction were due to real antibodies. The Wassermann tests were done by ice-box incubation with alcoholic heart extracts, not cholesterin reinforced—a method we believe to be of greatest delicacy, yet entirely safe. Dr. Swift, in his recent communication to the Academy, expressed the opinion which we have held for some time, that plain alcoholic extracts used in this way act as delicately as the cholesterin reinforced.

Dr. Lapowski: The studies upon spirochætes enlarge our knowledge of the spirochæte; but when a rabbit, inoculated with a pure culture of spirochætes, is referred to as a syphilitic, instead of an inoculated rabbit, the readers assume a far too important rôle for the spirochæte and the rabbit in syphilis. That the spirochæte is *the* cause of syphilis is not as yet proven, and our present knowledge of the relation between the spirochæte and the rabbit rather tends to question the conclusions drawn from such experiments, as to the syphilitic nature of the disease produced in rabbits. The points which have been usually mentioned to prove the specific nature of such inoculated lesions—their histological resemblance to lesions of human syphilis; the positive Wassermann reaction in the rabbits inoculated with spirochæte cultures; the appearance of syphilis-like lesions in places far removed from the place of inoculation—cannot be any more cited in support of the pathogenicity of the spirochæte. The Wassermann reaction is found in normal rabbits and is not considered any more a specific reaction. We must be careful in our estimation of the keratitis parenchymatosa, which occurs in inoculated rabbits, as it has been shown that plain horse serum, injected into a rabbit, produces exactly the same lesions as described under the name of keratitis parenchymatosa, and which are referred to by ophthalmologists as "keratitis anaphylactica." What is of the most importance is the demonstration of the fact that, in 24 per cent. to 30 per cent. of fresh rabbits, taken directly from their farm breeding places, papular ulcerations on the preputium, on the peno-scrotal and anal regions were present and that these lesions contained spirochætes which could not be distinguished from the *pallida*, either by the dark field examination or by Giemsa staining. It is therefore advisable to be careful in identifying the findings in the studies of spirochætes in rabbits with human syphilis, even if the readers of the papers are ready to consider the proposition that rabbits in their normal existence suffer from syphilis.

Dr. Field: Dr. Lapowski has mentioned that 30 per cent. of rabbits have spirochætes. Has anyone demonstrated spirochætes in 30 per cent. of rabbits? The statement also that rabbits have often a positive Wassermann reaction is negatived by most of us who have tested our amboceptor for any positive reaction. We have not found it. In 200 rabbits I have found one which gave a positive reaction. The proposition of finding spirochætes in rabbits and that of calling them *pallida* are two entirely different matters.

Dr. Lapowski: Dr. Field should make himself acquainted with the published reports of other workers in the same field. Regarding the positive Wassermann reaction in normal rabbits I can refer him to the observations of Fleischman, Fischer, Blumenthal and Dohi (*Neisser's Beiträge,* 1911, pp. 515–520) and to the statement of Professor Zinsser in his remarks of this evening. As to the presence of spirochætes which cannot be distinguished from *pallida* in normal rabbits, I can only again refer him to the article of Dr. Arzt (*Wien. kl. Woch.,* 1914, pp. 788–1053) and add that clinically it is often impossible to distinguish in the oral cavity the *Spirochæta dentium* (?) and in the genital organs the *Spirochæta calligyra* from the *pallida.*

Dr. Zinsser: Dr. Park's point about immunization with virulent pallida is extremely important. One year ago we began to immunize with spirochætes from the testicles of infected rabbits, but we did not succeed in getting enough material to get convincing results. We hope in time to get enough material into the rabbit to make the experiment successful. In regard to dead or living organisms—we can deal with living organisms only from cultures. We cannot give the living virulent organisms because we would be likely to get a lesion.

As to Dr. Lapowski's question about the keratitis being syphilitic, as Dr. McBurney has stated, we have not made the claim that it is syphilitic, because we did not succeed in finding spirochætes in the lesion, but we think it is syphilitic because such lesions have been described and proven to be so by other observers. In regard to the spirochætes being *pallida,* I would like to refer Dr. Lapowski to the work of Uhlenhuth and Mulzer. These workers give several points which are strong evidence in this regard. Dr. Noguchi states that he has produced syphilitic lesions with cultivated organisms. Another point is that last year in Germany there occurred in a laboratory a case of syphilis, contracted by a laboratory diener from the fourth generation in rabbits. That to my mind is strong evidence and points to the logical conclusion that the experimental disease in rabbits is true syphilis.

A METHOD OF PRODUCING ANTIGEN FOR COMPLEMENT-FIXATION IN TUBERCULOSIS

H. R. MILLER, M.D., AND HANS ZINSSER, M.D.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.)

The writers have prepared an antigen for complement-fixation in tuberculosis for which they claim certain advantages over those prepared by previous workers, especially those of Besredka and of Petroff, and the modifications of Besredka's antigen of Bronfenbrenner and Craig. One of the writers (Miller) has especially studied the Petroff antigen in connection with the one reported this evening. There is nothing very original about the method of preparation, which follows closely the endotoxin extraction method previously applied by Besredka to typhoid bacilli and other organisms—namely the grinding up of a weighed amount of bacillary substance with salt, and its subsequent suspension in distilled water until isotonicity is obtained. The antigen so prepared is not anticomplementary in quantities as high as 1.0 c.c. and has given positive fixations with quantities as low as 0.02 c.c. It does not give positive Wassermann reactions with syphilitic sera, that is, it has failed to do so in all but one case out of 26, and tuberculosis could not be absolutely excluded in that case. A total number of 190 cases so far examined are reported. Of these 93 were clinically negative cases the diagnosis being based on careful examination by attending physicians of three institutions, and in all of them the fixation test was negative. In 24 of the 93 positive skin reactions were obtained, but these were adults without clinical signs of tuberculosis. On the other hand 89 cases gave positive reactions, and all of these were actively tuberculous, ranging in degree from the incipient to the very far advanced third stage. This communication is entirely in the form of a preliminary report, but the writers make it because of the regularity of the results in the hope that others will be stimulated immediately to try it.

Discussion:

Dr. Park: If I understood the paper rightly the results were absolutely clear-cut. Cases which were positive clinically gave positive reactions, and those which were negative or too slightly developed to be diagnostic gave no fixation. Thus it seems to me that the test simply corroborated (in most cases at least) what was known already. Apparently there were no doubtful cases in which suspicion was aroused by the reaction. Even this, however, is a great advance and may be perfected to pick up the more doubtful cases.

Dr. Alfred Meyer: I have been interested in the work of Dr. Miller on complement fixation, and I had a number of specimens of blood collected for him. The clinical details of the cases were in my possession, and the conclusions afterwards compared with the serological findings. Among the twenty-six cases one was in the tuberculosis ward at Mt. Sinai. The patient had a history of very severe hæmorrhages. The question arose whether the stomach or the lung was involved. The patient was rather thin, with indefinite lung signs, which I said were not proven tuberculous. There was I thought rather evidence of gravitation of stomach blood giving localized crepitation. The patient was not, however, transferred from my tuberculosis ward. She was kept quiet in bed. She never had fever. The von Pirquet I cannot recall, but she was twenty years old and its result was therefore not important. She had repeated severe hemoptyses while in the ward and the question arose of controlling these with artificial pneumothorax. This was not done immediately, but I said it should be done the next day. I was leaving the ward when one of the house staff came running to me saying that the patient had just had a third hæmorrhage of 20 oz. We then immediately made an artificial pneumothorax and there has been no recurrence of the hæmorrhages for the past eight weeks. A guinea pig injected with the expectorated blood has just been reported "no tuberculosis," and this agrees with Dr. Miller's findings.

This is only one case, but being on the side that Dr. Park criticizes, the one case illustrates the possible value in doubtful cases. This girl is probably not tuberculous, though one must admit that once in a while a positive case of tuberculosis will give no reaction in the guinea pig. The correspondence between the clinical findings and the complement fixation test are to say the least of it striking, even though the results of treatment would justify the view of the pulmonary origin of the blood.

Dr. Kessel: Several of these cases were taken from my service at the Montefiore Home. With two exceptions, all of these patients gave a positive reaction with Dr. Miller's test. The one patient who reacted negatively was a man who had been on the service for many months. He had various attacks of fever lasting from a few days to a few weeks. His sputum was repeatedly negative upon examination. A guinea pig inoculated with his sputum failed to develop tuberculosis. A marked dilatation of the arch of his aorta led us to make the Wassermann test, which was found strongly positive. His response to salvarsan and mercury treatment was a poor one. In view of Dr. Miller's positive findings in the definitely tuberculous patients, the negative complement fixation result in this case would urge upon us the diagnosis of pulmonary lues.

The other patient who reacted negatively was one who came to us from a tuberculosis sanitarium. He presented evidence at the right apex of a healed lesion, there being dulness and no râles.

In view of the fact that these two cases, one of pulmonary lues, and the other of healed tuberculosis, gave negative results, while all of the other patients examined from the Tuberculosis Service yielded positive results, I believe it fair to regard Dr. Miller's fixation test with considerable optimism.

GAS BACILLUS INFECTION WITH REMARKS ON GASTRIC ULCER

RANDOLPH WEST AND MARY E. STEWART

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.)

Studies were made of a gas bacillus brought from the French front, particularly with regard to its toxin formation, since Weinberg and Sacquépée have recently described a true toxin from an organism belonging to this group. Cultural and morphological examination placed the organism distinctly in the Welch bacillus group. Filtrates of anaerobic broth cultures killed guinea pigs but it was found that this action could not be attributed to a true toxin, since neutralization of the acid filtrate removed this toxicity. Suspicion that the toxic effect was due to the acidity of the filtrates led to the injection intraperitoneally and intravenously of acid solutions equivalent in acidity to the toxic filtrates of the cultures. The gas-bacillus infected guinea pigs, in the large majority of the experiments, showed gastric ulcers. Gastric ulcers were also noticed in the guinea pigs injected intraperitoneally and intravenously with acid. Gastric ulcer, without death of the animal, occurred regularly after intravenous injection of 4 to 4.5 c.c. of 1 per cent. acetic acid. The acid solutions were not as uniformly toxic as the acid filtrates, possibly because of the presence in the acid filtrate of butyric acid which is highly poisonous.

Discussion:

Dr. Libman: This paper is of great interest to me because in 1901 I drew attention to the probable importance in the production of the phenomena of disease of the acid produced by bacteria when grown in carbohydrate medium. At that time and later I noted a number of features of growth of bacteria in the presence of carbohydrate and serum that had been overlooked. The important facts are the following: Precipitation of proteid may occur. Pigment and smell production may be curtailed or inhibited. In media containing blood and carbohydrate, agglutination was sometimes produced and at times coagula.

One must believe that these peculiarities of growth in the presence of carbohydrate must be significant as regards infection in diabetics in whom there is usually 0.5 per cent. glucose in the blood. They may even be of importance in non-diabetics in whom the proportion of glucose in the blood is about 0.1 per cent. and in whom until a part is closed off from the general circulation there will be a new supply of carbohydrate.

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PATHOLOGICAL CHANGES IN THE LIVER IN CHRONIC PASSIVE CONGESTION[1]

R. A. LAMBERT, M.D., AND B. R. ALLISON, M.D.

(From the Pathological Laboratory of the Presbyterian Hospital, New York)

It is well recognized that the liver in some cases of extreme chronic passive congestion shows a more or less extensive central necrosis, while in other instances a great dilatation of the capillaries without necrosis is found. In many of the older textbooks, and in certain of the recent ones, the second type of lesion has received the greater emphasis. The disappearance of the liver cells about the central hepatic veins is attributed by most authors to a progressive atrophy from increased blood pressure in the dilated capillaries.

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[1] A more detailed paper on this subject will appear in an early number of the *Johns Hopkins Hospital Bulletin.*

Mallory, among others, has objected to this interpretation of the lesion, maintaining that the effect of increased blood pressure would be felt equally by all of the cells in the liver lobule. He regards the disappearance of the cells in the central portion of the lobule as always the result of a toxic necrosis associated in most cases with hemorrhage. The toxic factor, he suggests, is a terminal bacterial infection, so commonly observed in persons dying from chronic disease. He has supported this view by demonstrating the frequent occurrence of central necrosis or degeneration of liver cells, in a large percentage of cases of acute infection not associated with chronic circulatory disturbance.

The chief object of the present study was to determine, first, whether the two lesions referred to, which may be conveniently designated "dilatation atrophy," and "hemorrhagic necrosis," exist independently, and, secondly, to discover he factors that determine the presence of each.

The material for this study was selected from 500 autopsies at the Presbyterian Hospital, among which there were about 90 cases of well-marked chronic passive congestion.

It may be stated in the beginning that a careful study of the livers of these cases has convinced us that there are not *two,* but at least *five* types of hepatic lesion associated with chronic passive congestion. While it must be admitted that this division into so many groups is in some respects arbitrary, and that many livers present more than one type of lesion, we believe that the types described are sufficiently well defined for practical consideration.

The general characteristics of the five types, together with the form of cardiac or circulatory disorder with which they are most often associated, are given in the following table:

Classification of Lesions

Type	Character of Lesion	Diseases with which Lesion is Most Often Associated	Number of Cases	Per Cent.
I	Capillary dilatation, central or diffuse, with atrophy of liver cells. No necrosis.	Arteriosclerosis, myocardial fibrosis, chronic nephritis with cardiac failure, moderate cardiac valvular disease.	32	31
II	Capillary dilatation (often not marked) with central degeneration of liver cells. No hemorrhage or definite necrosis.	Same as type I.	17	16.5
III	Marked central fat accumulation with mid-zonal capillary dilatation. Areas of hemorrhage and necrosis (type IV) often present.	Cardiac valvular disease, especially chronic rheumatic forms in young individuals.	9	9
IV	Central necrosis with hemorrhage.	Advanced cardiac valvular disease (especially cases with involvement of several valves), extreme myocardial fibrosis, arterio-venous aneurysm.	28	27
V	Central "collapse fibrosis." Lesion is usually secondary to type IV.	Prolonged circulatory disorder of any kind.	17	16.5

The separate grouping for the cases included in type III (central fat accumulation and mid-zonal capillary dilatation) may not appear warranted since an accumulation of fat is observed in many of the cases belonging to other types (especially II and IV). The gross and microscopic picture, however, is so strikingly different that we have felt justified in setting them apart. This particular type of lesion has been very fully described by several German authors.

While the relationship of these several types of lesion is not altogether clear, histological studies have, to a certain extent, made clear the sequence of changes. There can be little doubt, for example, that the "collapse fibrosis" (type V) is secondary to the changes described in type I or type IV, the disappearance of the liver cells taking place in the one case by a process of atrophy associated with capillary dilatation, and in the other by a more sudden necrosis with hemorrhage. We have studied several livers which showed very clearly transitions from type IV to type V. In the "collapse fibrosis" following hemorrhagic necrosis an enormous quantity of hematogenous pigment lying free or phagocyted by surviving endothelial cells is present. We have furthermore observed many instances of transition from type I to type II and type IV, also from type III to type IV.

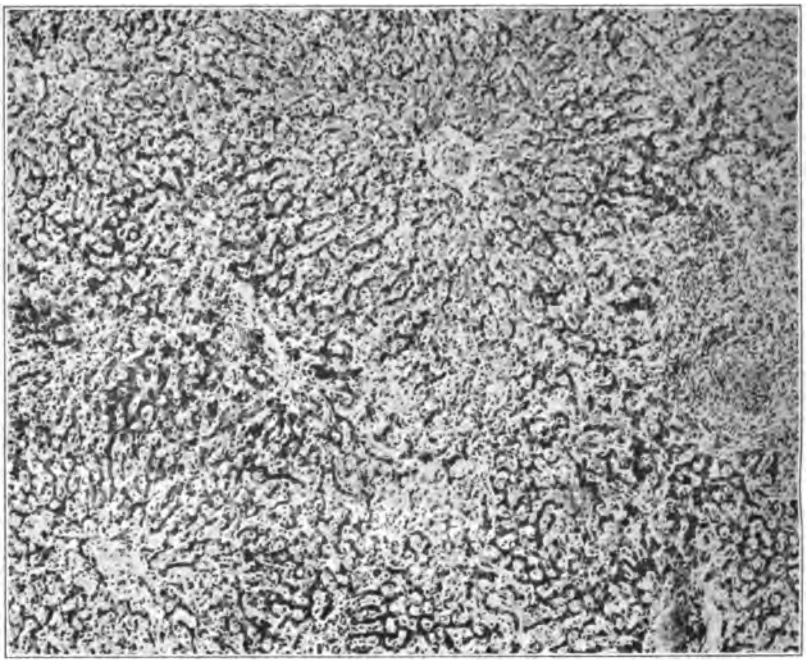

FIG. I. Chronic passive congestion of liver, type I, showing marked dilatation of capillaries with atrophy of liver cells. Case of arteriosclerosis, and chronic pulmonary tuberculosis, with a terminal generalized miliary tuberculosis.

It is clear therefore that the liver may pass through several stages of chronic passive congestion represented by at least three of the types mentioned above, ending in type IV or type V. It is equally clear, however, from histological studies, that in some cases a rapid and extensive necrosis has taken place, not preceded by any marked dilatation of the capillaries, since at the margin of the necrotic zone, which is often sharply defined, no dilatation of the capillaries whatever is seen.

In an effort to clear up the question as to why at autopsy one liver should show only capillary dilatation and atrophy of the central cells (type I) while in another case extensive central necrosis is seen, we have examined the clinical histories and autopsy protocols of the cases and tabulated them with reference to the following points: (1) The existence of a terminal complicating infection and (2) the severity of the lesion in the heart or vascular system, responsible for the circulatory disorder, for example, the degree of valvular stenosis or myocardial change, number of valves involved, etc.

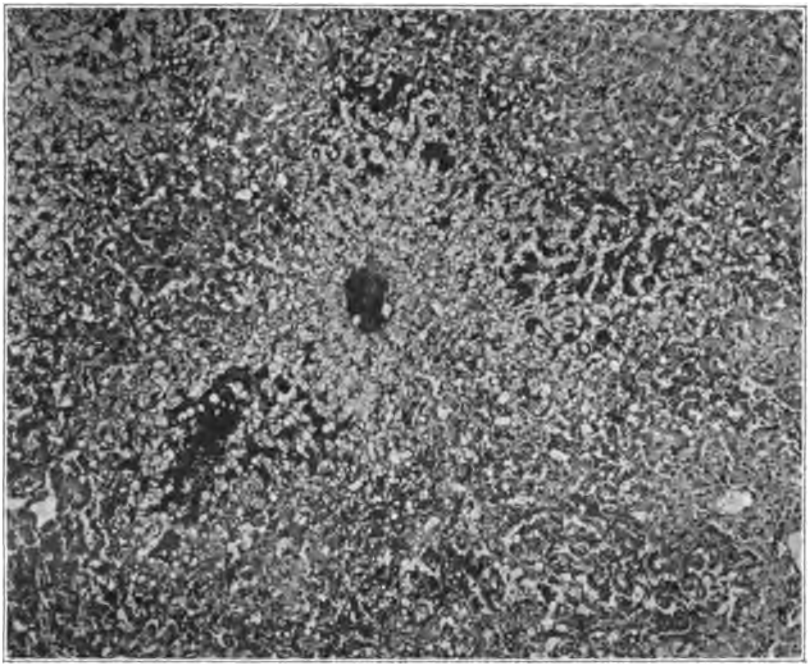

FIG. 2. Chronic passive congestion of liver, type III, showing marked central fat accumulation with mid-zonal capillary dilatation. Case of chronic rheumatic endocarditis, involving mitral, aortic and tricuspid valves; acute pericarditis and pleurisy.

An examination of this table has shown, first, that terminal infections were as common in cases belonging to type I, in which there was no necrosis, as in those of type IV, showing hemorrhagic necrosis. Cases in which there was a definite septicæmia were somewhat more common in type II (capillary dilatation with central degeneration).

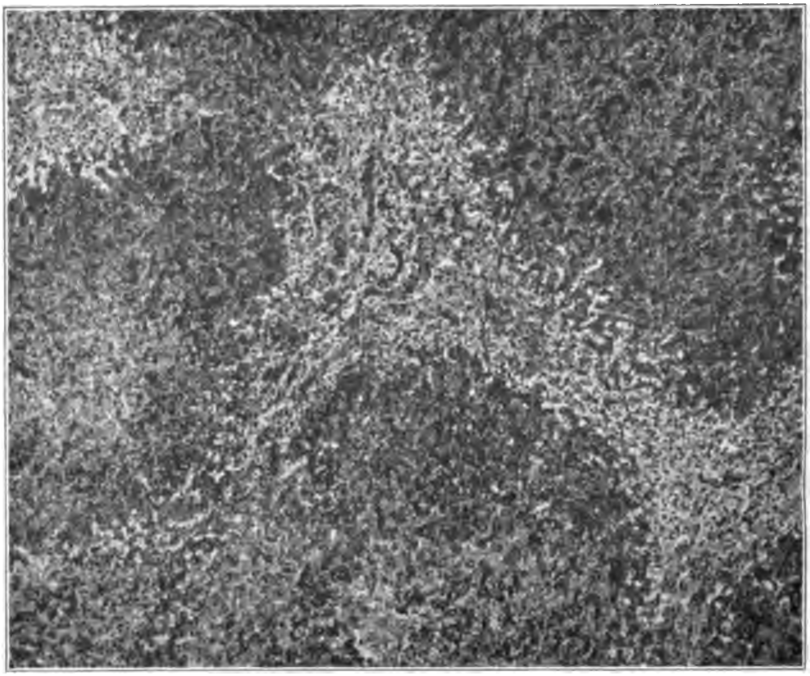

FIG. 3. Chronic passive congestion of liver, type IV, showing extensive central necrosis with hemorrhage and fatty infiltration of surviving peripheral cells. Case of myocardial fibrosis, generalized arteriosclerosis, adherent pericardium.

It was found, secondly, that in all of the cases with extensive central necrosis and hemorrhage (type IV), there was evidence clinically of extreme circulatory disorder, and that the anatomical changes in the heart or vascular system responsible for the chronic congestion, were very marked. For example, of the five cases selected as showing the most extreme necrosis, three were examples of chronic cardiac valvular disease with involvement of mitral, aortic and triscuspid valves; the fourth was a case of extreme mitral stenosis; in the fifth, where the most extensive lesion of all was present, the cause of the circulatory disturbance was an aneurysm of the arch of the aorta which had ruptured into the superior vena cava.

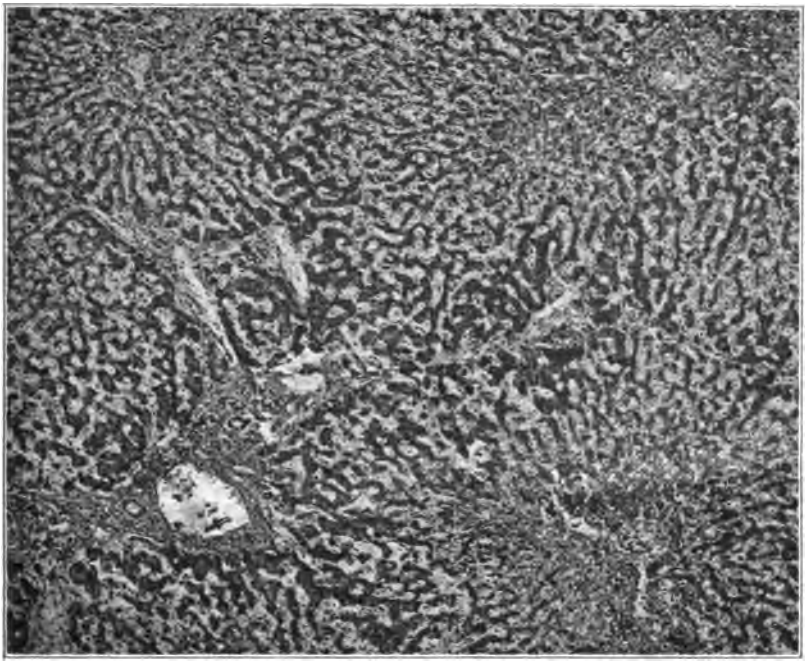

FIG. 4. Chronic passive congestion of liver, type V, showing central "collapse fibrosis." Case of aortic stenosis.

These findings would indicate therefore that the factor most responsible for the necrosis of the liver cells is the degree of circulatory disorder, and that terminal infections are of little or no etiological importance.

Discussion:

DR. PAPPENHEIMER: It is very striking to see how abruptly the necrosis stops in the hemorrhagic areas. I would like to know if Dr. Lambert has found any explanation that will throw light on this. On one side of the line there are perfectly normal liver cells; on the other there is just a mass of blood. The division is very sharp.

Dr. Wood: The more advanced lesions, especially the hemorrhagic, suggest a bacterial infection. Some of the experimental toxic liver lesions show extensive hemorrhage without necrosis; some show extensive necrosis without hemorrhage. It seems to me that the walls of the capillaries and sinusoids must be injured by some sort of poison to permit the blood to escape.

Dr. Lambert: Answering Dr. Pappenheimer's question, I think that the sharp line of demarcation between healthy and necrotic cells in cases of extreme chronic passive congestion, must in some way be referable to the peculiarities of the circulation in the liver with its two blood supplies under different pressures, meeting in the mid zone of the lobule. But I am unable to explain the exact mechanism of the process.

Regarding Dr. Wood's remarks, I do not believe it necessary to assume the existence of a toxin acting on blood vessel walls in order to explain the hemorrhagic character of the lesion. My idea is that a condition of marked stasis exists as the result of which both liver cells and endothelial cells suffer. After the disintegration of the liver cells, the blood of the engorged capillaries pours through the injured walls into the trabecular spaces. In other words, while the hemorrhage does result from an injury to the vessel wall, this injury is due simply to malnutrition or asphyxia of the cells as in hemorrhagic infarction.

A CONVENIENT STAIN FOR CONNECTIVE TISSUE

S. LOWENTHAL

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

In the staining of tissues great difficulty has been encountered in the attempt to bring out distinctly the fine connective tissue structures without obscuring the other elements. Of the various connective-tissue stains at our disposal those of Mallory and of Van Gieson are the ones most commonly used. A permanent and a very satisfactory connective-tissue stain is that of Maresch-Bielchowsky, but as the process is long and demands the undivided attention of the experimenter it is not used as a routine stain. Although Mallory's stain often gives satisfactory results after Zenker's fixation, it does not act uniformly, and owing to deterioration, a fresh solution of the stain must be made up frequently. Both Mallory's and Van Gieson's stains are apt to fade in time unless very carefully mounted.

The following method gives excellent results as a differential and connective-tissue stain and seems quite permanent, stained sections having retained their color for over two years. The process is very simple, and the results are uniform. Zenker's fixation is the only requirement.

Method: Tissues fixed in Zenker's are embedded in paraffin. After thin sections have been cut, they are fastened to slides and allowed to dry. The paraffin is removed, the sections are treated with iodine to remove the mercury, and with hyposulphite of soda to remove the iodine, and then washed thoroughly in several changes of distilled water.

Two solutions are to be kept in stock:

I. A saturated aqueous solution of basic fuchsin (3.5 gm. to 100 c.c. distilled water).
II. Aniline blue (Grübler) .... 0.5 gm.
Saturated aqueous solution of picric acid .... 100 c.c.

The staining process is as follows:

1. Place the sections in solution I for 5 minutes;
2. Wash off excess of stain in distilled water;
3. Transfer to solution II for from 15–30 seconds;
4. Dip in distilled water;
5. Dehydrate in 95 per cent. alcohol until no bluish color is given off;
6. Dip in absolute alcohol for a few seconds;
7. Clear in xylol and mount in acid free gum dammar.

Results: The connective tissue is stained a bright blue. The parenchymal cells of the organs take a green color, with the exception of mucous and preputial gland cells. The cells of the mucous glands take a pink stain in contrast to the green or bluish-green color of the serous glands. The preputial gland cells are stained yellow, and the cells of the sebaceous glands light blue. Nuclei, extremely cornified epithelium, and the intercellular substance of old hyaline cartilage, are stained red. Red blood corpuscles are stained yellow; striated and smooth muscle, epithelium, and nerve tissue are stained green.

This stain can also be used advantageously after formalin fixation. Sections which have been fixed in formalin are fastened to slides and are allowed to dry. The paraffin is removed and the sections are brought down to water. They are then placed in Zenker's fluid over night; the Zenker's fluid may be used with or without the acetic acid. The sections are then washed in water and treated as described above for the ordinary Zenker's fixation.

As far as can be ascertained there is no record of any other composite stain of this nature showing the same results, except that of Borrel; but Borrel's stain is difficult to use; it does not give uniform results; and it fades in some cases.

PRESENTATION OF SPECIMENS

A. B. EISENBREY, M.D.

(*a*) AN AMYLOID TUMOR OF THE LARYNX

The first case that I wish to report is one of an amyloid tumor on the left vocal cord of a woman sixty-six years of age, a patient of Dr. Voislawsky at St. Luke's Hospital. It was regarded as a neoplasm, possibly malignant, and was excised. We found that there was no evidence of actual neoplasm at all. The laryngeal mucosa was intact, but the underlying structures were heavily infiltrated with amyloid material. This was of more or less homogeneous structure but showed in some areas a lamellar arrangement. It was seen in the lymph spaces and loose in the tissues. Some of the masses showed a structure similar to prostatic corpora amylacea.

Few cases of amyloid tumor of the larynx have been described. Kaufmann alludes to three. Infiltrating and nodular types are distinguished. Amyloid degeneration in sarcoma and other neoplasms is very rare.

(*b*) TWO TYPES OF CARCINOMA OCCURRING IN THE SAME UTERUS

The second case is one of adenocarcinoma and epithelioma occurring in the same uterus. The patient was a widow fifty-nine years old, on the service of Dr. Mathews at St. Luke's Hospital. A hysterectomy was performed and the first specimen examined was diagnosed epithelioma. It represented a limited area but showed what appeared to be a small portion of adenomatous growth. In sections from higher up in the uterus an apparently separate and distinct adenocarcinoma was found. Sections through the entire posterior wall of the uterus show an epithelioma in the region of the cervix, and an adenocarcinoma in the fundus. The majority of cases which have been described as showing two types have been discarded on the ground that the portions described as epithelioma were merely areas of metaplasia in an adenocarcinoma which had the general appearance of squamous epithelium, on account of metaplastic changes in the glandular cells. I believe that there are two distinct growths in this case.

Discussion:

Dr. Pappenheimer: I would like to ask whether the amyloid in these localized amyloid tumors is usually found in the lymph spaces.

Dr. Eisenbrey: Yes, in the lymph vessels and in the walls of the blood vessels, and also, in the larger nodular form, in the tissue clefts.

Dr. Lambert: Do these amyloid growths give all the characteristic reactions of amyloid?

Dr. Eisenbrey: Sections of this tumor react to gentian violet.

Dr. Lambert: Do they react to iodine? Iodine and sulphuric acid reactions are obtained only in the later stages of amyloid change.

Dr. Wood: They did not give the iodine reaction.

Dr. Mandlebaum: In reference to multiple primary tumors of the uterus, one must be very guarded in establishing the diagnosis. The cases in which most uncertainty may exist are those which seem to show a squamous cell carcinoma of the body of the uterus, associated with adenocarcinoma of the cervix; for metastases from the cervix may occur, and if sufficient metaplasia is present, the secondary tumors may appear to be primary growths of squamous type situated in the body of the uterus. About ten years ago Hitschmann analyzed the cases that had been reported as primary multiple tumors of the uterus and seemed to prove that all of the reported cases were single tumors with metaplastic changes. In the case presented by Dr. Eisenbrey this objection can hardly be raised, for here the squamous type is in the cervix and the adenomatous type in the body of the uterus.

In looking up the literature some years ago I found that Klien in 1894 reported the first case of two tumors localized in the cervix, a squamous cell carcinoma of the portio, and a cylindrical cell carcinoma arising from an erosion in the cervical canal. This was probably the only case of multiple tumors of the uterus that Hitschmann could not dispute. In 1907 I had the opportunity of studying a similar case, which is, very likely, the second one of this kind on record. The specimen was obtained from a woman of fifty-one years with a large necrotic mass occupying the site of the cervix. Some of the sections showed pure adenocarcinoma, others squamous cell carcinoma, and, as a rule, both types were sharply separated.

In tumors of this kind situated in the cervix, three possibilities of interpretation must be considered. (1) The tumor may be unicentric in origin, arising from cylindrical epithelium and showing distinct metaplasia. (2) The tumors may be pleuricentric and spring from two separate anlages. (3) The tumor may arise from a single center of transitional epithelium where the flat cells pass over to the cylindrical type, and both types of tumor be produced. Even though two distinct types of tumor were present in my case, I hesitated to report it at the time, because a limited number of the cylindrical cells showed definite metaplasia. Dr. Cullen examined the slides and was inclined to think that the entire tumor was adenocarcinoma with metaplasia. Dr. Welch, on the other hand, agreed that the cervix was the seat of two distinct tumors.

DR. LEWISOHN: When I saw the announcement of Dr. Eisenbrey's presentation, I recalled to my mind a paper on metaplasia which I published about ten years ago. I reported two cases of metaplasia: (1) Cancroid of the pancreas, and (2) psammocarcinoma of the uterus. Both of these cases showed typical metaplasia. The transformation from cylindrical to squamous cell epithelium is very well demonstrated in the pictures which I pass around. There can be no doubt that in these cases we are dealing with true metaplasia. In many instances of two different types of carcinoma found in the same organ, the question whether the cause is a true metaplasia, or whether the apparent metaplasia originates from embryonal rests, is open to discussion. In my case of cancroid of the pancreas the latter possibility is out of the question, as this organ is in all its embryonal stages far removed from any ectodermal epithelium. In cases of double carcinoma of the gall-bladder a chronic irritation caused by stones has often been assumed to be the cause of the metaplasia. This is entirely out of the question in the pancreas case, as no stones were found. I would like to ask Dr. Eisenbrey if the tumors were very far apart, whether there was any possibility of a change in character of the cells from one kind to the other, or if such a possibility did not exist.

DR. EISENBREY: The areas were perfectly distinct in this case. The two types of tumor also were quite different. The adenoma was well up in the fundus; the epithelioma in the cervix. There were no cells of the squamous type to be seen in the area of the adenocarcinoma.

DR. WOOD: It seems to me from my study of the specimen that the two growths are distinct. I have seen many instances of metaplasia; apparently it is of frequent occurrence in old women. There is also squamous-cell hyperplasia—a sort of leucoplakia of the uterus. In this case there seems to be no point of contact between the two tumors and the difference in type is preserved. I have seen carcinomata of two types in the same uterus which metastasized and in the metastases preserved the two types.

Dr. Mandlebaum: In the study of a large series of tumors of the bladder I found five cases, mostly in elderly subjects, of typical squamous-cell carcinoma. A chronic inflammation such as cystitis, or a long-continued irritation due to the presence of a calculus, may result in an area of flat epithelium from metaplasia. If a carcinoma arises from this site it will naturally be squamous cell in type.

Dr. Wood: The case of the laryngeal tumor is the first that I have seen. It is necessary to be rather cautious in making a diagnosis of amyloid tumor in tissues about the larynx, as some fragments of thyroid gland, the colloid of which may be mistaken for amyloid, are not infrequently distributed in the tissues surrounding the gland or even at a considerable distance from it.

A SUPRARENAL ADENOMA IN THE MOUSE

SHIGEMITSU ITAMI, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

A search of the literature reveals only a few cases of hypernephroma in the mouse (Tyzzer, Haaland), all of which were situated in the kidney; the present example, so far as is known, is the only one in which such a tumor has involved the adrenal itself.

On June 28, 1915, a brindle female mouse (440/0), weighing 26.5 grams, was received at the laboratory; it appeared to be old, though its exact age could not be ascertained. A tumor in the left axilla, 2 × 1 × 1 cm., which proved to be an adenocarcinoma, was extirpated one week later. The mouse died, without recurrence, on November 12.

At autopsy, the left adrenal gland was found enlarged to about half the size of the kidney, and surrounded by an extensive hemorrhage; both kidneys and ureters, as well as the right suprarenal, were normal. The enlarged adrenal with its corresponding kidney was fixed in Zenker's fluid. As the amount of material was very small, no attempt at transplantation was made.

Microscopic examination of serial sections showed that the portion of the capsule facing toward the kidney was torn and hemorrhagic. The cortex was stretched, and compressed against the capsule, by a growth occupying the central region of the gland and appearing to originate in the medulla.

The tumor was clearly defined from the cortex, which it showed no tendency to invade. It was composed of solid alveoli, separated from one another by delicate connective-tissue strands and thin-walled capillaries. The individual cells were of moderate size and polyhedral shape, possessing rather small, vesicular nuclei and a well-stained protoplasm. No mitotic figures could be found. Though the cells resembled much more closely those of the medulla than those of the cortex, it is difficult to draw an exact conclusion regarding the site at which the tumor arose, though it probably sprang from the medulla. The resemblance to human tumors of similar origin was very close.

The comparatively regular arrangement of the cells composing the neoplasm, their fairly uniform shape and size, the expansive growth, and the absence of mitotic figures, all combine to suggest a diagnosis of adenoma.

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Discussion:

DR. PAPPENHEIMER: Did I understand the doctor to say that the cells were probably medullary. The Zenker gives a reaction with chromaffin tissue.

DR. ITAMI: The tumor looks like human adenomata in the adrenal; some of these arise from the cortical tissues, others from the medulla. We were not able to get a chromaffin reaction.

DR. PAPPENHEIMER: Is it not rare to find a tumor containing chromaffin tissue arising only from the medulla? I think this is exceptional.

A CASE OF SENSITIZATION TO WITTE'S PEPTONE

K. R. COLLINS, M.D.

The following case may be of interest on account of a very definite sensitization to peptone developed in a laboratory worker. I am not aware of such a case having been reported before. The facts are as follows:

The patient, Mr. M., twenty-five years old, in apparent good health, was employed in the laboratory of the Georgia State Board of Health from September, 1913, to September, 1915. Previous to this period he served in the army, and in April, 1911, was given typhoid vaccine. In June, 1914, he decided to take typhoid vaccine again. One half dose was given to him. This vaccine was grown in broth. Within a few minutes after the administration of the dose the patient complained of a choking sensation and became markedly cyanotic. The condition passed off in a few minutes, but on account of the severity of these symptoms subsequent inoculations were omitted. Five months after this second attempted immunization to the typhoid bacillus the patient complained of an irritable throat, and upon examination the Vincent's bacillus was found to be present. About this time he found that he was sensitive to peptone. A few minutes after handling peptone he would complain of constriction of the throat, and then develop a coryza and conjunctivitis, and a marked rash upon the face, principally about the eyes. The eyelids became swollen and edematous. On one occasion after weighing out peptone the attack was extremely severe, and the symptoms of collapse were so marked that strychnin was administered. He was then instructed to refrain from handling peptone in any way, but it was found that he could not even go into a room where peptone was being weighed or where a bottle of peptone was open. All peptone was then handled in one department, and he was instructed to keep away from this department. It was found also that the inhalation of the fumes of boiling broth after the peptone had been added developed this same condition, though the preparation of the broth up to this point did not affect him. He remained free from these attacks as long as he was not exposed to peptone. Witte's peptone only has been used in this department, and in all other departments of the laboratory until quite recently. I do not know whether he was exposed or not to the American peptone which is now being used in some cases.

On two different occasions, about one hour after the attack began, his nose, throat and eyes were examined by Dr. A. W. Stirling, occulist, who gives the following report: April 25, 1915, Pharynx—slightly granular. May 11, Eyes (right)—chemotic conjunctiva; (left)—same to a less degree. Nose—slight serous discharge. (Had used a little chloroform.) Glottis—evidently was edematous from the description of symptoms, but no evidence of this at time of examination.

Attacks were not brought on by inhalation of the dust from hay or of any other substances than the peptone. A part of his duties was the inoculation and bleeding of horses and the handling of guinea pigs, and he remained unaffected while doing this work.

Later he began to run an afternoon temperature and tubercle bacilli were found in his sputum. He was then advised to give up the laboratory work, which he did in September, 1915. His temperature has since become normal and the tubercle bacilli have apparently disappeared from the sputum, but a large number of Vincent's bacilli remain. My object in mentioning the Vincent's bacillus is that as we have had quite a number of sore throats, apparently due to this organism, it is possible that the first irritation of the mucous membranes of the nose and throat permitted the absorption of peptone.

I would like to call attention to the fact that Mr. M. had been in the laboratory and had handled peptone a year before he gave any signs of sensitization to this substance.

Discussion:

DR. EISENBREY: I know of a dean of a medical school who is sensitive to rabbits. He has an anaphylactic attack if he goes near these animals.

DR. WOOD: I have seen a man who is sensitive to mice, and for this reason was compelled to leave a laboratory in which he was employed.

DR. HOPKINS: This is apparently an instance of acquired sensitization to peptone, though I do not believe it is possible experimentally to sensitize animals to albumoses or peptones. The extreme sensitiveness of this patient resembles more the natural sensitiveness that Dr. Longcope is inclined to classify separately from the experimentally or spontaneously acquired type. The fact that the patient was not susceptible to peptone when first observed would, however, exclude him from this group.

A MODIFICATION OF RÖMER'S INTRACUTANEOUS METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF DIPHTHERIA ANTITOXIN IN BLOOD SERA

ABRAHAM ZINGHER, M.D.

(From the Research Laboratories of the Department of Health, New York City)

A convenient, practical and economical test to determine the diphtheria antitoxin content of blood sera is important, in that it would help to give us a solid scientific foundation in many experimental and clinical problems connected with the subject of diphtheria. The older subcutaneous test of Ehrlich for determining the antitoxin content by the death of the guinea pig has been universally accepted as a standard, but it cannot be used with any degree of accuracy, where less than 1/20 of a unit of antitoxin per c.c. of serum is to be determined. A method, therefore, that would be accurate, and enable us to determine as little as 1/200 unit of antitoxin, require but a small amount of serum, and be saving in animals, would be of considerable advantage.

In 1909 Römer and Sames[2] published a method for estimating small amounts of antitoxin in bloodsera. They determined the necrotizing dose of a toxin, *i. e.,* the amount of toxin which added to one unit, or a fraction of a unit, of antitoxin, was still capable of producing a slight local necrosis in the guinea pig. This was called the Ln dose of the toxin. Römer and Sames thus found that 1/500 M. L.D. of the toxin was sufficiently neutralized by 1/40,000 unit of antitoxin to just produce a slight local effect in the animal. This amount of antitoxin in 1/20 c.c. of undiluted serum (the quantity used in the test with an equal volume of toxin solution) represents 1/2000 unit of antitoxin per c.c.

The method suggested by Römer and Sames has not given reliable results in the hands of some other workers who have tried it repeatedly. The difficulties lie, first, in their attempt to make too fine a distinction between the various lesions of close lying tests. They thus claim to be able to distinguish a 5 per cent. difference in antitoxin content between the individual tests. A second point is the long time, twenty-four hours, required for the toxin and serum to stand before injection into the animal. Thirty minutes has proven to be a sufficient time to allow for the full union between toxin and antitoxin, even in the high dilutions used in the test. A third factor lies in the necessity of establishing a new toxin test-dose, the Ln. These factors have undoubtedly discouraged the use of a test which is inherently valuable.

The modification of Römer's method which I shall describe has been found simple and workable in testing many hundreds of human and animal sera. The L+ dose of toxin is taken as the standard and the toxin is so diluted that each cubic centimeter represents 1/100 L+ dose. A small amount of this dilution is mixed with an equal amount of whole serum, or of serum diluted with salt solution, and 0.2 c.c. of the mixture is injected intracutaneously into a guinea pig. In a series of such tests, made with graded dilutions of a given serum, two points are determined: the test containing the smallest amount of serum at which no lesion develops, and the test containing the largest amount of serum at which a distinct lesion appears. Within these two limits lies the antitoxin content of the serum. If the series changes abruptly from no necrosis to marked necrosis, an intermediate test is made. For example, if there is slight necrosis at "one unit" (toxin 1 : 100 + serum 1 : 100) and no necrosis at "one half unit" (toxin 1 : 100 + serum 1 : 50), the antitoxin content is one unit. If necrosis is marked the test is repeated for three quarters of a unit.

The method presents a number of advantages. It saves animals in that four tests can be made on each guinea pig. It also saves time in that the tests can be interpreted in 48 to 72 hours. As little as 1/200 unit of antitoxin can be determined in a serum with a fair degree of accuracy. If a distinct lesion appears at this test the serum is considered as having no antitoxin.

The details of the test are as follows:

1. The test-toxin. A standard, well-ripened toxin is used, so diluted with normal saline that 1 c.c. represents 1/100 L+ dose. A fresh dilution is prepared for each set of tests, as shown in the accompanying table.

2. The serum to be tested is used either undiluted or diluted 1 : 10 (0.2 c.c. serum + 1.8 c.c. saline), 1 : 100 (0.2 c.c. of serum dilution 1 : 10 + 1.8 c.c. saline), etc. The volume of serum used in the test is always 0.2 c.c., except where the test is made for 1/200 unit of antitoxin, when 0.4 c.c. of undiluted serum are added to 0.2 c.c. of toxin solution.

3. Normal salt solution is added to balance the mixture.

4. After the addition of toxin, serum and salt solution, the mixture is allowed to stand for 30 minutes at room temperature before injecting it into the guinea pig.

5. The guinea pigs (300–350 gm.) are prepared by removing the hair from the abdomen. This is best accomplished by simply pulling it out, leaving a central line of hair to divide the abdominal surface into two halves.

6. An animal holder is quite essential for accurate work and facilitates the exact placing of the injections.

7. A 1 c.c. "Record" or tuberculin syringe and a fine steel or platinum-iridium needle are necessary.

8. Four injections are made into each guinea pig, the abdomen being for this purpose divided into a right upper (R.U.), right lower (R.L.), left upper (L.U.) and left lower (L.L.) quadrants. The injections are made as far apart as possible to avoid a fusion of the lesions. Two tenths c.c. of each test-mixture is injected intracutaneously.

9. The places of injection on the guinea pigs are examined at the end of 24, 48, 72 and 96 hours and a careful record is kept of each test. The following changes in the local appearance of the skin at the site of injection are noted:

(*a*) *Redness* whether marked or slight.

(*b*) *Induration* whether marked, moderate or slight.

(*c*) *Necrosis*, marked or slight or only superficial scaling. The induration is most marked at the end of 48 or 72 hours, the necrosis begins to appear at the end of 72 hours.

INTRACUTANEOUS ESTIMATION OF DIPHTHERIA ANTITOXIN

This table shows the amount of toxin solution used, the dilution of the serum, and the quantity of normal saline added to balance the mixture. The figures in the right hand columns indicate the number of units of antitoxin per c.c., in a serum which prevents the production of more than a slight necrosis when mixed with toxin in the proportions indicated.

Test Toxin 1 c.c. = $\frac{1}{100}$ L+	Normal Saline	Serum				
		Undiluted 0.2 c.c.	1 : 10 0.2 c.c.	1 : 100 0.2 c.c.	1: 1000 0.2 c.c.	1:10,000 0.2 c.c.
0.2 c.c.	0.0 c.c.	0.01 unit	0.1 unit	1 unit	10 units	100 units
0.4 c.c.	0.2 c.c.	0.02 "	0.2 "	2 units	20 "	200 "
1.0 c.c.	0.8 c.c.	0.05 "	0.5 "	5 "	50 "	500 "
2.0 c.c.	1.8 c.c.	0.1 "	1.0 "	10 "	100 "	1,000 "

CONCLUSIONS

1. An intracutaneous method is presented for estimating, in an economical way, small amounts of antitoxin.

2. The test is easily carried out. It requires for accuracy, however, a uniform technique in making the dilutions of toxin and serum, the allowance of a uniform time for the two to combine, uniformity in the method of injection into the guinea pig, daily observations of the animals, and uniform interpretation of the tests.

3. This test is of value in experimental work in diphtheria, and in the accurate serological control of actively immunized individuals.

Discussion:

DR. HOPKINS: I should like to ask about the quantitative relation between this and the subcutaneous test. Is the L + dose the same as the Ln?

DR. ZINGHER: The two tests do not exactly correspond. The L + dose of toxin is very convenient for use. It represents a definite amount of toxin, which can be carefully established, and the results of the intradermal tests correspond quite closely with those of the subcutaneous test.

PATHOLOGICAL FINDINGS IN A CASE OF OCHRONOSIS

B. S. KLINE, M.D.

A case of ochronosis with melanuria was recently reported by Dr. B. S. Oppenheimer and Dr. N. W. Janney before the Section on Medicine of the New York Academy of Medicine. At present only a brief report of the pathological findings in this case will be given.

The patient, aged 40, male, presser by occupation, was admitted to the Montefiore Home and Hospital on November 18, 1915, having the clinical signs of ochronosis together with those of valvular disease of the heart. The patient died December 11, 1915, and was sectioned thirty-three hours after death.

PATHOLOGICAL REPORT

The autopsy revealed the lesions typical of ochronosis which will be but briefly described at this time.

The body is considerably emaciated. The skin in general is thin, sallow in appearance; in the arm pits it has a strikingly purplish-blue color. The cartilages of the ears are intensely blue in the inner portions, less intensely colored peripherally. The scleræ are tinted faintly blue except just below the cornea on each side where a much greater deposition of the pigment is present and in addition there is a wedge-shaped, greenish-blue area about 2 × 1 cm. The organs were removed through a rather small abdominal incision. The lungs, liver, spleen, pancreas, adrenals, bladder, seminal vesicles, and alimentary tract show no definite ochronotic pigmentation.

In the spleen there is a wedge-shaped, yellow opaque area reaching to the capsule about 2 × 1½ × 1½ cm. Near the apex of the infarct on section the tissue has a faint bluish tinge.

The heart weighs 360 grammes and shows dilatation of all the chambers with some hypertrophy of the right ventricle. At the base of one pulmonary cusp and at its attachment to the pulmonary artery in two places, there is a bluish pigmentation of the endothelium. At one place above the auriculo-ventricular ring, the endocardium of the left auricle is covered by numerous small friable warty vegetations. The aortic leaflet of the mitral valve shows on its upper surface a number of small gray friable vegetations. The posterior cusp is strikingly altered, there is a large, irregular, friable mass along the line of closure and free edge, yellow in color and in part calcified. The vegetation continues down the associated chordæ tendineæ. There is considerable bluish black pigmentation of the intima and endocardium along the line of attachment of the cusps of the aortic valve. This pigmentation is also visible on the posterior aspect of the aortic leaflet of the mitral valve. There are a few patches of bluish discoloration in the intima of the coronary arteries just beyond their origin from the aorta.

The kidneys together weigh 500 grams, are definitely enlarged, and each measures 13½ × 8 × 6 cm. The capsule strips readily, revealing a smooth surface on which the stellate veins are prominent. In addition there are innumerable pin point, or somewhat larger, bluish black spots. On section the cortex is uniform in width, averages 8–9 mm., has a watery reddish appearance, streaked and dotted everywhere with brownish and bluish looking pigment. This pigmentation, however, is most marked in the medulla, especially about the papillæ. The striations in the cortex are not very distinct but are apparently fairly regular.

The prostatic urethra presents a striking appearance; there are stony bluish-black masses of pigment varying in size from pin point to grape seed, projecting into the lumen, in some places uncovered by mucosa. No areas of injection are present about these masses. The prostate is of average size and consistency. On section it contains a large amount of bluish-black pigment varying from pin point granules to several each as large as a pea.

The aorta shows numerous atheromatous streaks in the intima and at just about the commencement of the thoracic portion there is a region of more advanced sclerosis in which for an area of a sq. cm. there is considerable bluish-black pigmentation.

The tracheal and bronchial cartilages appear bluish through the mucosa. On section, however, they appear brown. The costal cartilages present a striking appearance; they are hard and show an intense bluish-black pigmentation. The xyphoid also shows deep blue pigmentation. The ribs themselves show no apparent discoloration. The intervertebral discs show intense bluish pigmentation, they are more narrow than normal and in part invaded by bony tissue. The bodies of the lumbar vertebræ appear flattened. Macroscopically, the anterior ligment of the spine does not show any pigmentation.

Owing to permission for partial autopsy only, the larger joints could not be investigated. X-ray pictures of all the joints of the body, however, were taken. The spine shows changes characteristic of arthritis deformans. The larger joints (hip, knee) show well-marked changes, especially about the attachments of the capsules. The smaller joints show very little change (Scholz).

Histologically, the cartilages show the presence of the pigment in the form of fine granules in the matrix about the cells, and also in the perichondrium. The pigment is present in the form of granules in some of the cells of the tubular epithelium of the kidney, a few glomeruli likewise show a number of large mononuclear cells within the glomerular sac, all filled with fine brown pigment granules. In the sections of the kidney there is some distortion of the striations. In areas there is an increase in the interstitial connective tissue, in some of which accumulations of round cells in considerable number are seen. In some of these areas and elsewhere the glomeruli have an altered appearance, the tuft is bound to the capsule in one or more places by connective tissue. The ochronotic pigment is present in more diffuse form in the deeper layers of the endocardium and of the intima, in the renal tubules and in the corpora amylacea of the prostate.

The lesions observed in this case correspond closely to those in previously reported cases of ochronosis.

Discussion:

Dr. Ewing: It would seem possible that the early stages of ochronosis may sometimes be overlooked. I am very glad to have seen this case as I have never had the opportunity of examining the organs in this disease.

Dr. Kline: Virchow thought that up to a certain point the pigmentation was a physiological process and that the ochronosis was merely an overstepping of the physiological limits.

Dr. Ewing: Is there no general clinical guide as to the type of patient in which this condition occurs?

Dr. Kline: Some of the clinical signs are: Pigmentation of the scleræ, cartilages of the ears and skin of the armpits. Associated with these are signs of arthritis of the larger joints and spine, and frequently alcaptonuria.

Dr. Wood: Some cases may be overlooked clinically. Do cases of carbolic acid poisoning show alcapton in the urine?

Dr. Kline: In some cases the urine appeared normal. In some there were present phenol derivatives combined with ethyl sulphuric acid.

Dr. Zingher: What was the cause of death in this case?

Dr. Kline: Death was dependent upon the endocarditis.

ACTION OF RADIUM ON EMBRYO HEART MUSCLE

FREDERICK PRIME, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

During the course of a large series of investigations upon the action of radium on various tissues, it was found necessary, in order to complete the work, to observe this action upon embryo heart muscle. For this purpose the heart muscle from the chick embryo was selected as the most suitable, the points favoring its use being too well known to need more than a passing word. The ease with which it is obtained and kept living through an unlimited number of generations has been described at great length by Carrel and others; the fact that small pieces of this muscle will continue to beat for many days and weeks also had an important bearing on our work.

Our method was a simple one. The hearts were removed from chicks which had been incubated for seven days. They were then cut into small pieces, about 0.005 gm. in size, and suspended in a hanging drop of Ringer's solution, sealed over a deep slide. In each drop some twenty-five bits were placed. One of these preparations was kept for control observations, while the others were covered with 0.4 mm. brass and exposed to the action of 100 mg. of radium for one-half and two hours respectively, these exposures having been found in other experiments which have been reported at length elsewhere, to be a sub-lethal and lethal dose when, as was the case here, both beta and gamma rays are employed. Each piece of tissue was then removed and planted in chicken plasma slightly diluted, to which was added an extract made from whole chick embryos. These were kept in an incubator at 37° C.; daily observations were made; and at the end of forty-eight to seventy-two hours the bits were removed from the plasma, washed in warm Ringer's solution, and replanted in fresh plasma and extract in which they would continue their life cycle. The results of the observations made and collected from a large series of these experiments, extending over many months, are quite consistent.

On studying the cultures at the end of twenty-four hours, there was found to be a profuse growth of all the plants made in vitro, whether treated or controls, and the muscle in all cases could be seen to beat most actively. Often it seemed that those tissues treated with a short exposure of radium showed a more profuse growth than the controls, but further observations will be needed to determine whether this is or is not a constant result. At forty-eight hours the growth was slightly better than at the previous observation, but the pulsations were not so active as they had been. The tissues were then washed and transplanted; and the second generation was studied at the end of twenty-four hours. The growth in these was found to be good in all the cultures and there was no marked difference between the treated specimens and the controls. The number of fragments which were pulsating, however, was generally less than in the original cultures. In forty-eight hours these were again transplanted and on observation twenty-four hours later there was seen in this third generation quite a marked difference. The controls still showed, as was to be expected, a good growth, and the same was observed in the cultures treated with a sub-lethal dose of radium; but in the cultures which had been exposed to the radium for two hours, there was a marked reduction in growth. As a rule, there would be found one or two specimens which produced a slight growth; but in the vast majority of cases there was no growth at all, though many of those which showed no power of proliferation would still be beating, proving that they were at least alive. In the fourth generation no growths were found where a large dose had been administered, but still some fragments were pulsating; and in this generation there was a change in those cultures which had had a small dosage of radium. The specimens which showed growth were not so numerous nor were the growths which were present nearly so profuse as were those observed in the controls, and many plants could be seen to beat which showed no signs of growth.

In the fifth generation there was found to be practically no growth in any of the treated cultures, though many were beating; whereas the controls were growing and many more of them were found to be beating than of the radiumized specimens. From this point up to the ninth generation there was no change seen. The controls were growing and pulsating in many cases, while occasionally one was observed to beat without any growth; but growth was never found in the radiumized tissue, though several were observed to beat. When there was no longer any growth or pulsation, the tissues were discarded.

These results coincide very closely in most respects with the observations recently made and published by Dr. Wood and myself, in which it was found that a two-hour exposure to the beta and gamma rays killed tumor tissue so that it was unable to proliferate, whereas a half-hour did not always kill but simply retarded the power of growth. In the embryo heart, which is much more delicate than tumor tissue, it is seen that several days elapse before the action of the radium upon the proliferative power of the cells can be noted, and that finally the growth of the cells is inhibited, although the tissue itself it not killed. These observations correspond in many ways to those made by von Wassermann, in 1914, on the action of radium on tumor cells in mice. He concluded that the cells were not killed, but were rendered incapable of multiplication and that the apparent growth was an ameboid outwandering of cells whose power of mitotic division had been destroyed. Halberstaedter, in the same year, found that by radiumizing trypanosomes in vitro without much filtration, he could eventually prevent them from reinfecting mice. Hertwig stated that the most important part of the destructive action of radium was on the nuclear mechanism of division and not upon the cell protoplasm, and showed the effect of radium upon the nuclear division in ascaris eggs. His views also are confirmed by our observations.

Discussion:

DR. WOOD: It is interesting to realize that large doses of radium can be given to a cell which is capable of functioning but does not normally divide, such as heart muscle; while the same dose to epidermis or cancer tissue in which rapid division is occurring will kill the tissue. This is one more evidence of the importance of the function of the nucleus in the question of radium action, as was long ago shown by Hertwig. Radium does not seem to destroy the cell body except in overwhelming doses. In ordinary cell life division takes place, when the dimensions of the cell have increased its bulk so that the relation of surface to cubic contents has diminished to such an extent that the necessary absorption of food stuff is interfered with. The cell then divides in order to increase its surface. Otherwise it would die, and this is what happens to tumor cells which have been given a lethal dose of radium.

Another interesting fact is that if you radiumize a cell in the resting stage, you stimulate it if a sufficient dose is given; whereas the same dose will kill the cell if the nucleus is undergoing mitotic division. This seems to be the explanation of the great variations in the destroying effect of radium, as observed in human and animal tissues. In some cases the tumor will show an extraordinary response to the lethal dose of radium; in other cases no such effect is observed. The most susceptible tumors are those in which rapid growth is occurring, if you exclude certain types such as basal cell epitheliomata in which the cellular balance between growth and regression is very delicate. After the death of the cells which occurs from the inability to divide, their absorption takes place and then we notice the radium effect.

DR. KLINE: Is there anything characteristic about the histological appearance of the cell destroyed by radium?

DR. WOOD: There is nothing especially characteristic in the morphology of cells killed by radium any more than by any other physical agent. The paralysis of the mechanism of nuclear division results in the formation of multinucleated or syncytial cells. These are very commonly met with in radiumized tissues, but are also found in tissues which have been *x*-rayed, and occasionally about areas of necrosis produced by other changes. Both radium and *x*-ray are apt to cause an extreme proliferation of the vascular endothelium in the smaller arteries, which sometimes enables a diagnosis to be made.

Dr. Bowles: I would like to ask Dr. Prime how he exposed the tissues to radium.

Dr. Prime: The heart of the young chick was suspended in a drop of Ringer's solution. It was then exposed to the radium, which was filtered through brass, which permits the beta and gamma rays to go through.

THE GROWTH OF HETEROLOGOUS TUMORS IN THE CHICK EMBRYO

HOLLAND N. STEVENSON, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

In the course of experiments upon certain biological phases of transplantable mouse and rat tumors, it was found advisable to extend the work to include the growth of these tumors in the chick embryo. This paper reports some of the interesting points demonstrated in the experiments. The technique for opening and inoculating the eggs was the same as that employed by Murphy, who first showed that heterologous tumors could be grown with success in the membranes of the chick embryo. It was not found necessary to use eggs from a single strain of fowls, as the tumors seemed to grow equally well in eggs from all strains. Incubation was maintained at about 103° F. The embryo was inoculated between the fifth and the eighth day of incubation, and the graft removed for transplantation between the sixteenth and the eighteenth day. The tumor was cut into bits of 0.01 gm. and inoculated through the opening in the shell with a pair of curved forceps.

Of the eggs inoculated, between 35 per cent. and 40 per cent. were found when opened to contain living embryos. Of those living, 50 per cent. to 75 per cent. bore tumors, the percentage usually varying quite widely with the series.

The tumors inoculated into the membrane of the chick embryo with resulting growth were the Crocker Fund rat carcinoma 10, mouse carcinoma 11, and mouse sarcoma 180, also the Ehrlich mouse sarcoma, and the Buffalo rat sarcoma. The Ehrlich sarcoma was one of the tumors grown with success by Murphy. All these tumors grew well in the membranes of the chick embryo, and some have passed through several generations.

On opening the inoculated egg on the eighteenth day, the graft was found usually attached to the allantois near the site of inoculation; it was covered by the membrane which had grown around it and projected down into the allantoic cavity. In size, the graft varied from 2 to 6 mm. in diameter; it was quite firm and on section was translucent throughout, rarely showing areas of necrosis. On comparing it with the same tumor grown in mice or rats for an equal length of time, it was seen that the growths were about the same in size. The tumors in the animal, however, almost always showed some necrosis.

Microscopically, there was evidence of active growth. The tumor cells were seen wandering out into the mesenchyme tissue of the membrane, the sarcoma cells coming out singly, but rarely far from the main growth. The carcinoma cells extended out in groups, maintaining their general arrangement as in the original tumor. There was practically no change in the general appearance of these tumors during growth in the chick. The sarcoma cells possibly appeared larger; but this was very probably due to the difference in their immediate surroundings, for the mesenchyme tissue of the chick was much more delicate than adult connective tissue and pressure on the tumor cells must have been much less. Mitotic figures were present in abundance. A round cell reaction was always found around the growing tumor in the chick membrane, although this varied considerably in the number of cells present.

It was thought advisable to determine whether the characteristics of the Ehrlich sarcoma were modified in any way by a sojourn in the chick embryo. For this purpose a number of eggs were inoculated, and at the same time and from the same tumor, inoculations were made into a series of mice. The mouse series was used as a control and the growths were transplanted on the same day that transplants were made into mice from tumors growing in the chick embryo. It was found, on inoculating mice with a tumor grown in the chick embryo for one generation, that the number of takes was smaller than among the control animals, but that the average weight of the tumors was greater than of those in the control series. On transplanting these tumors from mice into the next generation of mice, the number of takes was the same as in the controls and the average weight of the tumors almost the same. This showed that growth in the chick embryo had influenced this tumor in some way, but that this influence ceased on subsequent transplantation into mice. With a tumor grown for two generations in the chick embryo and then transplanted into mice, the number of takes was less than in the controls and also much less than in the mice inoculated with tumors grown for one generation in the egg. The average weight of the tumors of this series was much less than that of the tumors in the control series. This indicates more strikingly that growth of the Ehrlich sarcoma in the chick embryo influences its behavior when it is reinoculated into mice.

Discussion:

Dr. Weil: Is there any theory to account for this loss of virulence on retransplantation back into the original host? Murphy has retransplanted tumors but I do not think that he has made this observation. Has Dr. Wood any theories?

Dr. Wood: It is very interesting to speculate as to why the first generation of inoculations from egg to animal is larger than from animal to animal. The increased growth may be due to the fact that the piece which is transplanted from the egg is composed of actively dividing cells, as the lantern slides have shown, while the fragments of tumors direct from the animal often contain but few mitoses. It may be therefore that the piece from the egg gets a start, so to speak, which accounts for its larger size; but the second generation from animal to animal will go back to its normal growth rate as Dr. Stevenson has shown. The lowering of the rate of takes may be due to the fact that the rapidly dividing cells when removed from the chick embryo are more easily damaged by the trauma; and possibly the fact that the cells have fed upon chicken proteid instead of upon mouse proteid may render necessary a certain amount of readaptation to conditions of growth in mouse tissue.

LIMITATIONS TO THE CULTIVATION OF MAMMALIAN TISSUES IN VITRO

R. A. LAMBERT, M.D.

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A number of enthusiastic papers on the subject of tissue cultures, for some of which the writer was responsible, have undoubtedly give rise to erroneous impressions as to the possibilities of the method, especially regarding the variety of mammalian tissues which may be cultivated. It was, I believe, with the idea of correcting certain of these misconceptions that the President of the Society suggested the present report of my experience with the method.

I should like first of all to present a classification of tissues based on their behavior in vitro. Afterwards the factors which may possibly be responsible for failure in the propagation of certain of the tissues will be briefly considered.

Our experience has suggested that tissues may be grouped as follows:

1. Tissues showing no cell multiplication or migration in cultures. Microscopic sections indicate that the cells in the tissue fragments survive for a time. Examples are: highly specialized cells, such as liver and kidney epithelium, and ganglion cells; also those of human malignant tumors.
2. Tissues showing no multiplication but active wandering. The cells die and undergo disintegration after a few days' activity. Examples are: leukocytes, mononuclear wandering cells of spleen, lymph glands and bone marrow and epithelium of skin and cornea.
3. Tissues showing both active multiplication and migration which cease after a short time in spite of efforts directed toward continued propagation. Examples: malignant tumors of rats, mice, and fowl.
4. Tissues showing active multiplication and migra-

tion with tendency to progressive increase of growth in subcultures. Example: connective tissue cells of all species.

It is clearly important to know the normal behavior of various tissues in culture, as otherwise erroneous interpretations of experiments are apt to be made. Spleen, for example, was used by Carrel and Burrows,[1] in studying the influence of physical changes in the plasma medium on cell activity. The apparent stimulating effect observed has been referred to as an "activation of growth." Since the cells which migrate during the first two or three days in spleen cultures, are leukocytes and mononuclear wandering cells which show no evidence of multiplication, it is obvious that there could have been no true stimulation of growth.

Why certain tissues fail to grow and others exhibit only a brief period of growth in vitro is a question not readily answered.

Examining the list of tissues given above it is to be noted first that many specialized epithelial cells show only a brief survival. This is not surprising in view of the recognized susceptibility of such cells in the body to mechanical and toxic injuries, or even to mere disturbances in nutrition. Indeed it is rather remarkable that such sensitive elements as ganglion cells should survive for any considerable period. Also the temporary activity of leukocytes and related cells of the spleen, lymph glands and bone marrow, is not to be wondered at, since the life of these cells in the body is probably very short, and they do not reproduce themselves even in vivo.

On the other hand, it is surprising that the cells of malignant tumors of rats and mice should show only a brief period of activity in contrast to the prolonged, apparently unlimited, growth of connective tissue cells of those species; also that the cells of human malignant tumors cannot be cultivated by methods which allow the growth of normal cells and benign tumor cells.[2]

In seeking the explanation for these differences in the behavior of tissues in vitro several factors may be considered: mechanical injury, accumulation of toxic metabolic products, presence of inhibiting antibodies, and nutritional disturbances.

Mechanical injury. This factor is certainly important in determining success in the cultivation of any tissue, as small experience with the method will show. But much evidence could be submitted, especially regarding the rat and mouse tumors, to prove that tumor cells are not particularly susceptible to traumatic injury. Besides, such injuries would explain only the failure of the cells to grow in primary cultures and would not account for the early cessation of growth observed.

Accumulation of metabolic products. In tissue cultures, put up according to the method in common use, there is no mechanism for the removal of waste products, or for the continuous supply of fresh nutritive medium. It is conceivable, therefore, that the accumulation of the products of metabolism may exert an inhibiting action on the growth of very active cells. In order to eliminate this factor as far as possible, early transfers have been made and the tissue fragments have been washed for varying periods in salt solution or hypotonic Ringer's solution as recommended by Carrel[3] and his co-workers who attributed their success in the prolonged cultivation of embryonic connective tissue largely to this procedure. We did not find that this treatment changed our results. It may be stated in this connection also, that, in the cultivation of adult connective tissue, an increasingly active growth was observed when the tissue fragments were transferred to fresh medium without washing.

It has been concluded, therefore, that the factor of inhibition by metabolic products does not serve to explain the failure of certain tissues to grow, or the limited growth of others.

Presence of inhibiting antibodies. While it has been established by various methods that antibodies for tumor cells do not exist in the circulating blood of rats and mice, it has been more difficult to settle this question for human tumors. Freund and Kaminer[4] have reported the discovery in human blood serum of two substances affecting tumor growth; one in the blood of patients suffering from cancer which exerts a protective influence on the cancer cells; the other in non-cancerous individuals which is lytic for cancer cells as demonstrated by test-tube experiments. While the experiments of these authors have not received confirmation by competent investigators, their work cannot be entirely disregarded.

In earlier experiments we used, as a matter of convenience, blood plasma from several normal individuals as a culture medium for human tumors. The negative results obtained and the work of Freund and Kaminer noted above, suggested that the plasma from the tumor-bearing individual might prove a better medium. It also occurred to the writer to see whether the presence of isoantibodies, agglutinins and hemolysins, influenced the growth in vitro of either tumor or normal tissue. This study has shown first that human carcinoma and sarcoma cells are not readily cultivated in vitro even in plasma taken from the individual carrying the tumor, while normal human connective tissue cells and benign tumor cells (mixed tumor of parotid) grow as well in homologous as in autogenous plasma. Furthermore the presence of isoantibodies is without demonstrable effect.

Nutritional disturbances. Before discussing this factor it may be well to consider the question advanced recently by Burrows as to whether cells growing in vitro can metabolize the food stuffs in the plasma medium. Burrows has expressed the view that the cells do not get nutriment from the medium at all. He assumes that they live on food carried over in the cell cytoplasm, a condition analogous to that seen in many developing ova.

In my opinion, a number of observations make this view untenable. In the first place, it is obviously impossible that a sufficient quantity of nutriment could be present in the cell cytoplasm to maintain active multiplication and wandering for any considerable period, whereas we know that active life in vitro, in the case of connective tissue cells at least, can be maintained for weeks, months or possibly years (Carrel). In the second place, if the cells did not take up nutriment from the medium succeeding generations should show a progressive diminution in size; but careful observation proves that this is not the case. Thirdly, the growth of cells in salt solution or greatly diluted plasma, where the food supply is markedly reduced, is of short duration and the cells of the second and third generation do become smaller. They show also very little fat accumulation as the result of the reduction in the quantity of this food stuff in the medium.[5] It therefore must be admitted, I think, that cells do take up nourishment from the plasma in which they are growing.

If the failure of certain cells to grow is to be attributed to the food supply, the trouble must lie either in an inadequate supply or in the absence of certain specific food stuffs. That the quantity of nutriment is adequate is indicated by dilution experiments with those tissues which can be cultivated. Practically all such tissues will grow in plasma diluted with equal parts, or more, of salt solution without any effect on the rate or duration of growth.

Regarding the second possibility, namely, the absence of special food stuffs, it may be suggested that connective tissue cells can be cultivated for long periods in plasma of foreign species.[6] This result is opposed to the idea of strict food specificity. However, in the hope of supplying some such necessary hypothetical substances, extracts from various organs including liver, spleen, thyroid, testis, malignant tumors, and adrenal, have been added to the culture medium. No effect from such additions was observed.

CONCLUSIONS

Connective tissue is the only mammalian tissue which can be cultivated in vitro for any considerable period.

The transplantable tumors of lower animals show very active growth for a short time only. The previous reports of prolonged cultivation of such tissues were probably due to confusion between tumor cells and connective tissue cells in the culture preparations.

Human malignant tumors have not been cultivated successfully by us, although human connective tissue cells and wandering cells show marked activity in cultures.

The limited growth of animal tumor cells and the negative results with human malignant tumors may be referable to several factors, mechanical injury, accumulation of metabolic products, presence of inhibiting antibodies, and nutritional disturbances; but my experience leads me to believe that no one of these factors is alone responsible.

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Discussion:

Dr. Wood: A presentation of the difficulties in the growth of tissues in vitro such as Dr. Lambert has just given is most wholesome. Judging from the majority of the published reports, the growing of tissues, including cancer, is extremely easy; but in my laboratory we have found that human and animal tumors, on the contrary, grow with great difficulty despite the most careful technique. A full knowledge of the difficulties may lead easily to some improvements in this important biological method.

Dr. Ewing: At Buffalo, some years ago, some possible limitations of the growth theory of tissue cultures were mentioned. Although the presence of growth phenomena was not denied, it was suggested that the appearances might represent, in part, out-wandering of cells and prolonged survival.

Dr. Lambert: I understood Dr. Ewing to express the opinion at Buffalo that there was no growth of tissues in vitro. I have not taken that position at all. There is very active growth of certain tissues; some tissues show only migration of cells and others show only a temporary active growth. I emphasized the difficulty of cultivating human malignant tumors.

Dr. Ewing: I did not suggest at Buffalo that there was no growth in vitro but that the phenomena observed might be referable to a survival and migration of cells.

Dr. Wood: The real difficulty is to get continuous growth. It is easy enough to get some proliferation for a few days, the cells probably growing on their reserve supply of nutrition; but when these cultures are transferred to fresh plasma they usually refuse to grow further.

THE ANALYSIS OF SERUM SICKNESS

RICHARD WEIL, M.D.

It was suggested some years ago by von Pirquet that serum sickness is in some manner due to the interaction of antigen with newly formed antibody, in the blood. This theory was supported by the observation that precipitin may be demonstrated in the blood of the patient toward the end of the serum sickness. Two years ago I was able to show that these precipitins possessed the function of passively sensitizing normal guinea pigs toward antigen,[1] thus demonstrating that anaphylactic antibodies play a rôle in the reactions of this disease. In addition, I was able to show that the antigen itself, namely horse serum, can be demonstrated in the blood even after the serum sickness has subsided. Within the past year I have studied a series of cases of serum sickness with the object of determining the presence of precipitin and of precipitable free antigen in the blood at various stages of the disease. Antigen was demonstrated by the reaction of human serum with the serum of a rabbit immunized against horse serum. The results of these studies have been summarized as follows: Preceding the serum sickness antigen is present in large amounts; the antibody is not demonstrable. Either at the beginning, or one or two days after the onset, of the serum sickness, antibody becomes demonstrable and increases in amount steadily for a period of one, or even two weeks after the serum sickness has subsided. Following this, the antibody is demonstrable in the blood for weeks or months. Antigen is present throughout the serum sickness and persists for a week or more after its subsidence, and then disappears. Thus antigen and antibody are both present in the blood of the patient during the manifestations of serum sickness and persist together for a week or more thereafter. Finally, the antibody alone remains, the antigen having entirely disappeared.

Parenthetically, it should be added that our present conceptions do not permit of the view that the factors which coexist in the serum correspond in the strict sense of the terms to precipitin and precipitinogen. In view of recent studies[2] such an interpretation is impossible. Horse serum is composed of a considerable number of antigenic substances, and as each one of these is thrown out of the serum by the accumulation of the corresponding precipitin only those partial antigens remain to which antibody has not yet been formed in excess. In accordance with this view, it is found that serum taken from the patient in the third week after the therapeutic injections gives a precipitate with serum of the same patient taken in the second week, although the former itself contains partial antigens not yet neutralized by corresponding antibody.

The question arises, how do these observations bear upon serum sickness. It seems evident, in the first place, that the phenomenon cannot be due, as originally suggested by von Pirquet, to the interaction of antigen and antibody in the blood, inasmuch as both of these two factors are present for some time after the subsidence of the sickness. The second possibility is the interaction of the cellular antibody with the antigen. Such an interpretation would be in harmony with the theory that anaphylaxis is invariably a cellular phenomenon, and, moreover, with the observation that human cells possess antibodies which respond to the antigen exactly as do those of the guinea pig.[3] The cellular antibody might react either with cellular or with circulating antigen in the production of serum sickness. The time relationships permit of a probable conclusion upon this point. It is known from previous studies that neutralization of cellular antigen by cellular antibody begins about six days after the introduction of the antigen, and continues for a week or ten days. At the end of this period the cellular antigen has been destroyed. These facts, to which I drew attention in 1914,[4] have been thoroughly elucidated in a paper by Dr. B. S. Denzer which will shortly appear in the *Journal of Infectious Diseases*. It is evident, therefore, that the period of serum sickness closely corresponds with the period of neutralization of cellular antigen by cellular antibody in the guinea pig.

The question naturally arises, why circulating antigen does not unite with cellular antibody to further prolong the serum sickness. The answer to this question is found in the fact that the union of cellular antibody with free antigen, when it takes place gradually, and especially when the cellular antibody is already partly saturated, gives rise to no symptoms. On the other hand, the explosive combination of circulating antigen with cellular antibody produces a sharp anaphylactic response. In one of the cases studied in my series this reasoning was sustained by the following circumstance. The patient, who had received a series of therapeutic intraspinal injections for meningitis, and had recovered from the ensuing serum sickness, developed a recurrence of the symptoms of the disease, requiring a fresh injection. Blood taken just preceding this injection revealed the presence both of antigen and of antibody. The fresh injection was at once followed by a recurrence of the serum sickness. Unquestionably, the sudden introduction of a large amount of antigen led to an explosive combination with the cellular antibody. The same process also explains the so-called "accelerated reaction" to antigen on the part of individuals who had, at some previous time in their history, received a dose of the same serum, and therefore had in their bodies cellular antibody which promptly responded to a fresh introduction of the antigen.

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ACUTE GLOMERULAR LESIONS FOLLOWING INJECTIONS OF BACTERIA INTO THE RENAL ARTERY

A. M. PAPPENHEIMER, M.D., H. T. HYMAN AND F. D. ZEMAN

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In the course of work planned to study the reaction of a previously sensitized kidney to general infections, it became desirable to investigate somewhat more closely the fate of bacteria introduced in large numbers into the kidney by direct injection into the renal artery. These preliminary experiments, upon which we wish now very briefly to report, seem to us to have a bearing upon the relation of bacteria to the lesions in acute glomerular nephritis.

The organism used in most of the experiments was a hemolytic streptococcus kindly supplied by Dr. Zinnser from his laboratory stock. It was of low virulence for rabbits, and as much as two agar slants could be injected intravenously without causing obvious symptoms. Fine emulsions in normal saline, freed from coarse clumps, were injected directly into a renal artery with a fine-gauge needle. A successful injection is followed immediately by a general blanching of the kidney, the color returning almost instantaneously after the needle is withdrawn. Hemorrhage from the puncture wound is in most cases very slight. The temporary disturbance of the blood supply is well borne, and in only a few instances have the lesions observed been of such a character as to suggest that infarction had occurred.

The kidneys were removed at varying times after the injection of living or killed organisms, and the histological lesions and the distribution of the bacteria studied in sections. Without giving at this time detailed protocols, we may describe briefly the general results of our observations upon the fate of the injected cocci, and the lesions produced. It was hoped that we should be able with this technique, to induce changes of a more chronic character, comparable to the subacute or chronic glomerular nephritis in man. We soon found, however, that the extreme frequency of spontaneous nephritis in rabbits (about 33 per cent. in our animals) made it extremely difficult to interpret the lesions of a more chronic character, and to bring these into definite relation with the injected bacteria. So far as our experiments go, however, we have as yet been unable to reproduce the later stages of acute glomerular nephritis in rabbits. Our report, therefore, deals only with the acute changes occurring within the first few days after the injection.

One of the most striking points noted was the rapidity with which the injected organisms were taken up by leucocytes, and possibly also by fixed cells. In a kidney removed five minutes after injection, for example, numerous cocci were found in the sections, and most abundantly in the glomerular capillaries, each tuft containing one or several clumps or chains. A close study shows that even after this short interval comparatively few of these cocci lie free in the capillaries. They are found in or upon the cytoplasm of the polynuclear leucocytes circulating through the tuft. At times it appears as if the capillary epithelium also ingested some of the organisms, and this is undoubtedly true of the endothelial cells of the capillaries between the tubules. Cocci were found also in stellate connective tissue cells, and occasionally lying free in contact with the basement membrane of the tubules. None were found free in the capsular spaces of the tufts nor in the lumina of the tubules.

The opposite kidney, removed ten minutes after injection, showed the same picture. After fifteen minutes practically all the cocci appear to be intracellular, their distribution and number being about the same as before. At this stage histological changes, as one might expect, are insignificant, but one does find in some of the tufts an increased number of polynuclears.

After one hour following the injection of a living culture distinct histological changes are present. The cocci are still found in abundance, but they are entirely confined to the glomeruli. Many of the tufts, even with the low power, seem to be more cellular than normal; and this is found to be due to the presence within the capillary lumina of plugs of cells, principally polynuclear, often imbedded in a pink-staining finely granular matrix, which does not stain as fibrin, and appears to be composed of agglutinated platelets together with cell fragments. The organisms are practically all within these cells, and appear, many of them, to be undergoing intracellular digestion, as evidenced by their swollen and irregular shape and their imperfect staining. Aside from these cell fragment thrombi in the capillary lumina, only slight changes are observed. The capsular spaces are wide, and may contain a delicate granular coagulum, and occasionally a red blood cell or two. There is no severe hemorrhage and no exudation of leucocytes into the capsular spaces, nor is there any marked alteration in the epithelium covering the tuft or lining Bowman's capsule.

Two hours after injection of a similar dose very few cocci are present in any part of the kidney; those found are entirely intracellular and present only in the glomerular tufts. The lesions are similar to those observed after one hour, with the addition that occasional loops are distended with hyalin thrombi.

In a kidney removed four hours and five minutes after injection of streptococci, distinct lesions were found in many of the tufts. The accumulation of polymorphonuclears is intense. giving the tufts an extremely cellular appearance, and quite evidently impairing the passage of the red blood cells through them. The leucocytes, as in the previous kidneys, are confined to the tufts, and are not present in the capsular spaces, which are free from exudate. There is no desquamation of the epithelial cells. A few glomeruli contain hyalin thrombi in one or more loops, and about these the nuclei are distorted and fragmented. The glomerular lesions are diffuse, practically every tuft being involved to some extent. In spite of this marked inflammatory reaction very few organisms could be demonstrated. Those found were in short chains or appeared within the leucocytes of the glomeruli; a few small clumps also were found in the larger veins.

The left kidney, injected with an equivalent dose of killed streptococci, showed lesions of the same character affecting at least 90 per cent. of all the glomeruli; in some of the tufts there was noticed in addition a swelling of the capsular epithelium and of the epithelium covering the tuft. The lesion differed also in the absence of thrombi, and careful search failed to show stainable bacteria.

Seven and a half hours after injection of living cocci, diffuse and intense lesions of the glomeruli are found. Many of the tufts are practically bloodless and collapsed, in which case the distended capsular space is usually filled with a granular coagulum. The capillaries are crowded with leucocytes, some of which occasionally find their way into the capsular space. In addition there are found thrombi in one or several loops. There is also a slight periglomerulitis, especially where the thrombosed loop comes in contact with the capsular wall.

A kidney examined twenty-four hours after the injection of living streptococci shows lesions more focal in character than those previously described. Both in the cortex and in the pyramids, there are present definite suppurative foci with numerous organisms. About 60 per cent. of the glomeruli show a distinct lesion. These vary in intensity, affecting in some of the glomerular tufts only a single loop, in others the entire glomerulus and periglomerular tissue. In the most severe lesions the cocci form emboli completely filling the vessel, whereas in the less severely affected tufts the streptococci are present in small groups or chains, or even in single pairs. The slightest lesions noticed are hyalin thrombi distending a single loop of the tuft, with degeneration of the adjacent endothelial nuclei. The injury to the capillary wall at the site of the thrombus is evident from its swelling and hyalinization. The overlying epithelial cells may be desquamated, and where the thrombosed loop comes in contact with the capsular epithelial cells, the latter become swollen or cast off, and a definite fibrinous adhesion may be present. In other tufts there is a very dense accumulation of leucocytes both within the tufts, in the capsular spaces, and in the loose tissue about the capsule. There is no marked degeneration of the tubular epithelium, but in many places wandering cells are present between the tubules, and these may contain cocci.

The right kidney, which was injected at the same time with killed streptococci, also showed lesions of the glomeruli, but the reaction was less violent than in the kidney injected with living bacteria. There were no suppurative foci. Cocci are present in some of the glomeruli, in clumps of considerable size. There are, however, no definite bacterial emboli, and most of the organisms are definitely intracellular.

Since dead organisms could no longer be demonstrated after seven and a half hours, it seems probable that in this experiment living bacteria reached the right kidney through the general circulation. In another experiment the kidney, twenty-four hours after injection of dead cocci, showed relatively slight lesions.

The above observations seem to show that one can produce acute glomerular lesions of the intra-capillary type by direct injection both of living and killed streptococci into the renal artery. Ribbert, Baehr and Quimby, and Fitz have produced toxic nephritis by the injection of uranium nitrate, iodine, etc., into the renal artery, but, so far as we are aware, no attempt has been made to study bacterial lesions by this method, which enables one to bring large numbers of organisms into contact with the renal tissue. The experiments throw some light on the fate of the organisms which are thus brought to the kidney. Even with the enormous numbers of cocci injected, it is found that after twenty-four hours practically all the organisms are disposed of, and even after eight hours no organisms can be demonstrated. The only exception appears to be in the kidney removed after twenty-four hours, in which persistence and multiplication of the organisms seems to have occurred. In several kidneys removed at intervals of from two days to several weeks no such persistence of the streptococci was observed.

We shall not describe the lesions in these kidneys in detail at this time, because the occurrence of spontaneous lesions has made it difficult to correlate them with the effects of the bacterial injections, but it seems certain that with the organism of low virulence used in our experiments the disposal of the bacteria takes place very rapidly. We have attempted to determine whether a large proportion of the organisms were excreted through the urine. In a rabbit in which the left kidney had been removed several weeks previously, the right renal artery was injected with a thick suspension of living streptococci, and cultures made by aspirating the urine of the bladder at five-minute intervals. The maximum number of colonies was obtained twenty minutes after the injection, but the total number of organisms recovered from the urine during the first hour was not over 400. Toward the end of the experiment only ten colonies per c.c. were obtained. In a second similar experiment the urine was practically sterile throughout, but the secretion of the kidney was evidently much impaired by the long continued narcosis.

Although these results cannot be taken quantitatively as indicating the rate of excretion of the bacteria, the experiments show, nevertheless, that under these conditions relatively few bacteria are passed out in the urine. This agrees with the histological findings, in that no cocci are present in the capsular spaces or in the tubules. That many of the injected organisms pass directly through the kidney into the general circulation could be shown by taking cultures from the renal vein. Immediately following injection one c.c. of blood was found to contain innumerable colonies, and even after twenty minutes there were over 700 colonies per c.c. In a second experiment large numbers of streptococci were obtained in a culture from the renal vein made one hour and five minutes after the injection, so that the discharge of the organisms into the circulation appears to continue for a considerable period. It would seem, however, that the bacteria which are thus discharged into the renal vein do not continue to circulate in the blood but are probably retained in the lungs. Cultures from the ear vein, made about half an hour after the injection, show only a few colonies.

The bacteria which remain in the kidney appear to be largely deposited and arrested in the glomeruli. This is not due to mechanical obstruction, because they are frequently found here as isolated pairs or short chains which do not plug the capillaries. They are, however, rapidly taken up by circulating leucocytes, so that even after five minutes many of the bacteria are no longer free in the circulation. A few of the bacteria appear also to be taken up by the fixed cells of the tissues, being found in the endothelium of the capillaries, and also within the spindle shaped or stellate connective tissue cells between the tubules. This rapid phagocytosis would appear to explain the early disappearance of the organism from the lesions, and to make clear also why it is that it has always been so difficult to detect bacteria in acute glomerular nephritis, clinically of streptococcal origin.

Discussion:

DR. THALHIMER: The work of Drs. Pappenheimer, Zeman and Hyman is very interesting. I should like to mention in connection with this paper some work, which is not yet published, done by Dr. Baehr and myself about a year and a half ago. We used the same technic for injection of the renal artery as used by Dr. Baehr in his previous work on experimental nephritis. This technic is the same as that outlined by Dr. Pappenheimer. *Streptococcus mitis*, or *viridans*, was used in our experiments. The object of the work was to study the lesions produced by the streptococci injected and especially to learn if embolic glomerular lesions similar to those found in cases of subacute bacterial endocarditis are produced. In this latter we were successful. Streptococci were present in the portion of the glomerular tuft affected and remained there for a considerably longer time than did the hemolytic streptococci reported by Dr. Pappenheimer, *i. e.*, at least as long as nine days.

In a few other experiments which I performed *Staphylococcus aureus* or *Micrococcus tetragenus* were injected into either the portal vein or renal artery of dogs. These experiments were undertaken to find out if bacteria could pass through the normal kidney into the urine or through the normal liver into the bile. Occasionally a few bacteria were present in the bile but never in the urine. Incidentally it was found that the bacteria were present in great numbers in the general circulation for at least two hours after injection. They were therefore not filtered out by either liver or kidney.

I would like to ask Dr. Pappenheimer whether the hemolytic streptococci were found in his experiments in the glomerular tufts in the later stages of the lesions described by him.

DR. EWING: What was the final result in these cases? Was the picture of chronic nephritis ever produced?

DR. PAPPENHEIMER: In answer to Dr. Thalhimer's question, I am not able to say offhand whether organisms were present in these cases which showed thrombosis and necrosis of glomerular tufts.

Replying to Dr. Ewing I can only repeat what I said in my paper, that we are not ready to state what the final results of these lesions are. Any conclusion on this point has been especially difficult because of the common occurrence of spontaneous chronic nephritis in rabbits.

LESIONS PRODUCED IN SENSITIZED RABBITS BY INJECTIONS OF BACTERIAL EXTRACTS INTO THE RENAL ARTERY

C. B. COULTER, M.D., AND A. M. PAPPENHEIMER, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York)

The purpose of the following experiments was to investigate the influence of previous sensitization to bacterial proteins upon the appearance of renal lesions, when bacterial extracts are brought into direct contact with the tissues of the kidney.

Rabbits were used for the experiments, and were given ten daily inoculations of increasing amounts of bacterial extracts. The extracts were prepared, by grinding the dried bacterial sediment obtained from mass cultures with sodium chloride crystals and glass sand, and then bringing to isotonicity with distilled water. In some cases sodium carbonate was added to an amount of 0.2 per cent. The determination of the actual sensitiveness of the animals was attempted by means of intradermal skin tests, but the results were most disappointing, as control animals reacted for the most part in the same manner as the treated ones. No other tests were attempted, and it was decided to regard the change in the kidney itself as evidence of sensitization.

About fourteen days after the last sensitizing injection, the animals were anesthetized, and given an injection of the bacterial extract directly into the renal artery. Forty-eight hours later, the animals were killed, and the kidneys prepared for examination. The frequency of spontaneous lesions, radial scars, and round cell accumulations in our rabbits, made it seem wiser to limit our observations to acute lesions.

The first experiments were with typhoid bacilli. The bacterial extracts used were not sterilized nor filtered, from fear of altering the bacterial protein, and were freed from bacillary masses only by brief centrifuging. This left small numbers of free bacilli and small masses of bacterial fragments in the supernatant fluid used for the injection. The kidneys of two treated rabbits, injected with 0.25 c.c. of such extract, show after forty-eight hours in the injected kidney—the opposite being entirely normal—glomerular lesions, corresponding closely in distribution and extent to those described by Löhlein and Baehr in subacute bacterial endocarditis. One in every six or seven glomeruli is affected, and shows changes in only one or in several loops of the tuft. The affected loops are bloodless and contain a very finely granular material, in which, especially about the periphery, there are polymorphonuclear cells and nuclear fragments. Occasionally a small loop contains a homogeneous thrombus with only one or two leucocytes, while in other loops the polymorphonuclears and nuclear fragments are more abundant, and in some tufts there is merely a diffuse accumulation of the leucocytes. The endothelial cells enclosing the smaller cell groups can often be made out distinctly, in other cases their nuclei are pyknotic, and the cells appear to be a part of the degenerating cell mass. The *membrana propria* of some capillaries of this sort appears thickened and hyaline. The capsular epithelium does not show desquamation, and there is no exudate into the capsule. A very few capillaries outside of glomeruli, show similar changes to those within tufts.

Two control animals receiving injections of the identical extract died within forty-eight hours, and their kidneys were not examined. Similar amounts of two other typhoid extracts, made in exactly the same manner, were then injected into normal rabbits. In one the kidney appears entirely normal, in the other there are a few areas of polymorphonuclear accumulation in one portion of a glomerulus, and a single small loop was found presenting the same picture of nuclear fragmentation, etc., seen in the kidneys of the treated animals. There is a distinct difference in the extent and severity of the changes, from those in the treated animals.

The question at once arises, are these lesions merely embolic in origin, as claimed for those of subacute bacterial endocarditis? To determine this, rabbits were treated with typhoid, pneumococcus, and streptococcus extracts, and injected after an interval, as before, with bacterial extracts which had been centrifuged strongly, to free them from bacterial fragments that might act as emboli, and in which the bacteria had been killed. The kidneys of two typhoid, three streptococcus and five out of six pneumococcus rabbits so injected show no pathological change. It appears, then, that under the conditions of our experiment, the presence of bacterial bodies has been essential to produce the lesions.

It seems quite possible that the length of contact—not over thirty seconds—of the cleared bacterial extracts with the kidney tissue, was too brief to allow of any interaction between the two; and that the clumps of bacterial fragments in the extracts first used, while not acting as emboli, a conclusion suggested by the slight changes in the normal rabbits, at any rate allowed a longer contact and in more concentrated form, between the antigen and the tissues. To test this point and determine more sharply the influence of sensitization, we then sensitized a rabbit with a single injection of egg white, and, fourteen days later, at which time the rabbit gave a positive skin reaction, injected the left kidney with 0.3 gm. egg white dissolved in 1.2 c.c. of 5 per cent. gelatin solution. It was hoped that the viscidity of this solution would delay its passage through the kidney and allow a longer contact with the tissues. At the same time the possibility of embolism seemed to be excluded, as the fluid was perfectly labile at 37° C.

The kidney of a normal animal injected with this egg-white solution appears entirely normal. In the injected kidney of the sensitized animal were found distinct glomerular lesions, which have again the focal distribution of the endocarditic renal lesions. One in about every ten glomeruli was affected, with involvement of one or several loops. The affected loops are plump and bloodless, and bear a close resemblance to those in the typhoid kidneys, although the polymorphonuclear accumulation and fragmentation is less marked, and there is greater evidence of endothelial swelling and proliferation. The capillary lumen is often filled with, or compressed by, swollen cells, apparently those of the endothelium. Where a capillary loop or an efferent vein is filled with a thrombus, apparently of platelet origin, the accumulation of polymorphonuclears and the nuclear fragmentation very closely resembles that in the typhoid kidneys. Capsular exudate and proliferation of epithelium were not noted, although about some capsules were small round-cell accumulations.

It appears from these experiments that a local anaphylactic or sensitization reaction may be produced within the kidney by intrarenal injection, and that this consists, at one stage, of glomerular lesions, with the production of cell thrombi and endothelial swelling and proliferation. Their focal distribution in the case of the egg-white reaction is difficult to explain. We have not studied the later stages. The results obtained with our typhoid extracts, which are primarily injurious even if to only a slight degree, require further study before they can be accepted as conclusive. Further experiments with guinea-pigs are in progress, and we hope here to test for sensitiveness by producing acute anaphylactic death, and to employ the technic used in the egg-white experiments with cleared bacterial extracts.

THYROID AND PARATHYROID TUMORS OF THE TONGUE

F. C. WOOD, M.D.

(From the Pathological Department of St. Luke's Hospital)

The presence of masses of thyroid tissue at or near the base of the tongue, while of considerable surgical and scientific interest, is a rather infrequent occurrence, only about one hundred cases having been published up to 1916. For this reason I wish to report the following cases which have been observed at St. Luke's Hospital. All were in the service of Dr. Frank S. Mathews, whose permission I have to use the clinical records.

CASE I. H. O., female, age, 11 years. The child had always had a peculiarly husky voice from the time she learned to talk. About one year before admission she had a sore throat, and at that time her mother discovered an enlargement on the tongue, which had grown only slightly since. The child had never complained of pain or tickling in the throat, and except on the day of admission, when she vomited a little blood, there had been no record of bleeding. She was slightly defective mentally, but not myxedemic. Physical examination showed nothing of note, except that at the base of the tongue there was a rounded reddened tumor, seen best when the tongue was protruded. The growth was wholly attached to the tongue and apparently did not involve any other tissue. It measured about 2 cm. in diameter and when removed was found to be quite hard and filled with straw-colored fluid. There were similar collections of fluid in the wall.

When examined in the laboratory the specimen was found to consist of a cyst, with considerable connective tissue forming the wall. Microscopical examination showed the cyst to be composed of loose connective tissue with numerous blood vessels and some hemorrhages. Evidently the cyst was not a true thyroglossal duct remnant, but was merely a simple cyst of thyroid tissue. In the wall there were a number of nests of thyroid gland structures (Fig. 1), a small nodule resembling parathyroid, and a good deal of muscle, probably derived from the tongue.

The finding of chief interest in this case was the fragment of tissue resembling parathyroid. A photomicrograph of this material is reproduced with photomicrographs of sections from two normal parathyroids taken without altering the position of the microscope and lenses (Figs. 2, 3, and 4), so that the magnification in all is exactly the same. The close resemblance of the three is evident.

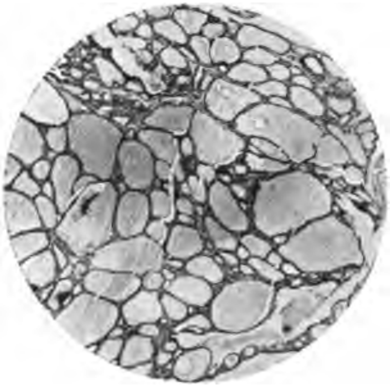


Fig. 1. Thyroid tissue from tongue.

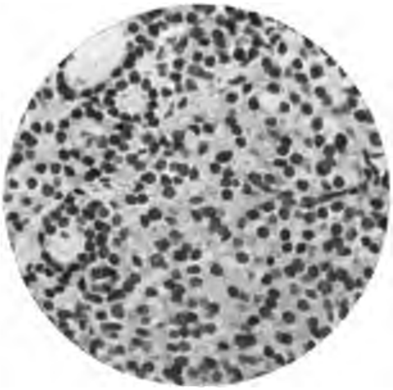


Fig. 2. Parathyroid tissue from tongue.

Unfortunately the tumor was fixed in formalin, so that no satisfactory demonstration of glycogen could be made.

Case II. A. S., female, age, 37 years. No history obtainable. At the level of the upper border of the thyroid cartilage there was a small, egg-shaped tumor, consisting of thyroid tissue, entirely separate from the main thyroid gland and not adherent to the surrounding tissue. At the base of the tongue, just posterior to the foramen cæcum, there was a small solid tumor about the size of a pea.

Microscopical examination of the piece of tissue from the tongue showed irregular masses of thyroid scattered throughout the connective tissue. At one portion, the acini were much distended and filled with colloid material; in other places the glands were small. An area resembling parathyroid occurred in these sections also.

The neck tumor measured 5 × 4.5 × 3 cm., and was smooth and round, and covered with a thin capsule beneath which were seen a few small blood vessels. Cut section showed firm, lobulated tissue, yellowish in color, which cut with a gritty resistance and presented numerous small lobules and acini. Microscopically the specimen was composed of typical thyroid gland tissue.

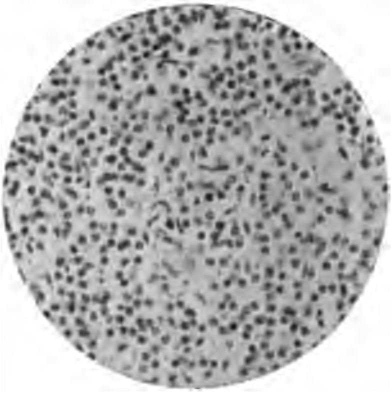


FIG. 3. Normal parathyroid.

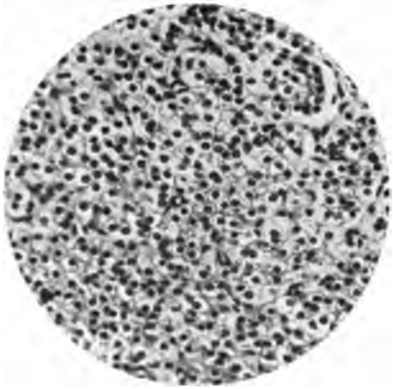


FIG. 4. Normal parathyroid.

These two cases are excellent illustrations of this rather rare condition. Neither have since developed myxedema.

A third case, which illustrates another phase of these tumors, is one in which the tumor was derived from the base of the tongue.

CASE III. H. W., female, age, 27 years. The patient had suffered from a lump in the neck ever since she could remember, which had remained about the same size and had given rise to no symptoms. On physical examination a small circumscribed and encapsulated mass was found on the right side of the neck at the level of the thyroid cartilage. This was freely movable. The thyroid was not palpable.

The specimen received at the laboratory was a cyst, measuring 2.5 × 1.5 × 1.5, roughly spherical, with a lobulated surface. Microscopical examination showed thyroid tissue with several large dilated acini surrounded by areas of thyroid tissue containing smaller acini. There are also remnants of a thyroglossal duct bearing ciliated epithelium (Fig. 5).

This type of tumor is much more frequent than that occurring on the upper surface of the tongue.

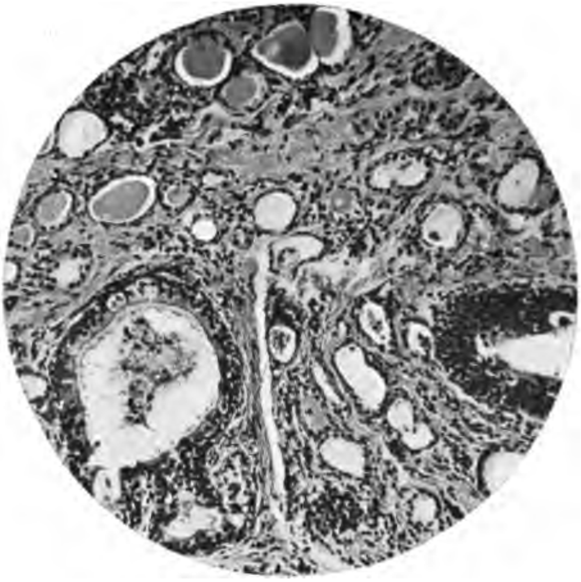


FIG. 5. Thyroid tissue about thyroglossal duct.

The following is a summary of the general facts known from examination of the cases heretofore published:

The usual site of the tumor is in the median line, occupying the area where the foramen cæcum is ordinarily situated. Sometimes, however, the thyroid tissue may be found at a short distance from this position on the dorsum or along the lateral border of the base of the tongue, but these cases are very rare. The tumor may be of small size, not over half a centimeter in diameter, but in some instances a mass the size of a lemon has been noted. It may scarcely project from the surface of the tongue or may appear as a lobulated, in some cases pedunculated, mass at the base of the tongue, which greatly interferes with swallowing or speaking. The surface of the tumor is covered with mucous membrane and is usually smooth. The color is often a dark red or purple, due to the great vascularity of the structures beneath the membrane covering the growth. Severe hemorrhages have been noted following injury by the teeth or food. The differentiation between cysts, papillomata, and the malignant tumors which rarely occur in this region is easy. Some of the thyroid masses may be very firm, though not so hard as the chondromata that may occur at this site. In addition to the pathological interest connected with these misplaced thyroid gland masses, it is of surgical importance to remember that in about one-tenth of the cases the thyroid tissue in the tongue is the only example of that glandular structure in the body. Therefore, if the tongue tumor is completely extirpated the patient will develop myxedema.

In a case reported by Asch,[1] a patient after extirpation of a tumor at the base of the tongue developed not only myxedema, but also tetany; and on microscopic examination a number of small bodies resembling to a considerable degree the microscopic structure of the parathyroid were found. The presence of parathyroid bodies is difficult to explain from an embryological point of view, for while the median lobe of the thyroid is chiefly derived from the tissue which goes to form the tongue and springs from the point of junction of the second and third branchial arches, the base of the tongue is formed by two projections from these arches, which meet and form the foramen cæcum and the lingual duct. The latter extends down into the neck under the hyoid bone and inside the median thyroid lobe. The duct structure is lined in its upper portion by squamous epithelium, and in its lower portion by cylindrical ciliated epithelium. It usually disappears, but when it persists it is frequently surrounded by more or less scattered masses of thyroid tissue. The parathyroids are of a slightly different origin; ordinarily they are seen to arise from the pouches of the third and fourth branchial clefts, which are in close relationship to the tissue from which the lateral lobe of the thyroid originates. The parathyroid bodies are usually found in or on the surface of the lateral portions of the thyroid gland itself, and parathyroids are not seen in connection with the central portion of the gland. A recent investigation of new material has suggested that possibly the second branchial pouch may give rise to some parathyroid tissue which would explain the presence of parathyroid in and about the tongue region.[2]

The only other interesting clinical feature attached to these tumors is that they occur almost entirely in women, only 12 per cent. being noted in men. Microscopical examination of the tissue of such masses shows either perfectly normal thyroid tissue with follicles filled with colloidal material or a rather rudimentary gland with follicles not containing any colloid, or a regular cyst-like cavity such as is seen in goitrous conditions of the thyroid gland itself. Hemorrhage may occur and softening, as is frequently seen in enlarged thyroids in the usual anatomical situation.

References:

1. Asch: *Deutsch. Ztschr. f. Chir.*, 1914, cxxx, 592.
2. Grosser, A.: Über die Entwickelung des vorderen Theiles des Kopfdarmes menschlichen Embryonen, Wien, 1911.

Discussion:

Dr. Coulter: I would like to inquire as to the embryological explanation of the presence of parathyroid tissue in this region so far removed from the normal situation of the parathyroid.

Dr. Ewing: I have just looked over Dr. Wood's section and think it is impossible to say whether one has to do with young thyroid tissue or parathyroid tissue.

Dr. Wood: While it is generally stated by embryologists that the parathyroids come from the third and fourth pouches, a recent author has claimed that they may develop also from the first pouch, citing in support of his view a case similar to the one reported to-night. I agree with Dr. Ewing that it is difficult to say whether we are dealing in this case with both parathyroid and thyroid tissue or with thyroid tissue alone. It may be pointed out, however, that it is hard to understand why some of the thyroid tissue resembles the adult type, while other tissue in the same specimen is distinctly embryonic. It seems likely that we have to do with both parathyroid and thyroid, as the resemblance of some of the tissue to normal parathyroid is very close.

THE BIOLOGICAL EFFECTS OF RADIUM ON TUMOR CELLS

FREDERICK PRIME, M.D.

(From the George Crocker Special Research Fund, F. C. Wood, M.D., Director)

There has been much discussion of late as to the penetrating power of radium rays, and the efficacy of these rays in the treatment of malignant growths situated at some distance from the surface.

Two series of experiments were undertaken to determine the effect of radium on animal tumors. In the first, the tumor to be radiumized was removed and exposed in a moist chamber to the radium. At the end of the exposure, these pieces of tumor, together with the control which had been kept under similar conditions, were inoculated into mice or rats, as the case might be. The tumors were charted at weekly intervals, and from the growths which appeared, successive generations were propagated. When the gamma rays were used either in small or in large doses, it was found that, after the first generation, the tumors which had been exposed to small doses of radium for a short time produced larger daughter growths than did the controls, larger also than those which had been exposed to a dose of radium just under the lethal point.

A second series of experiments was conducted upon tumors in vivo, a mouse carcinoma being used for this experiment. Here the mouse was anesthetized and the skin over the tumor reflected. Then after a small portion had been removed from the tumor and retained at the same temperature as a control, the radium, filtered through 0.4 mm. of brass was applied to the tumor. This filter passes the gamma and all except the softer groups of beta rays. Exposures to 83 mg. of radium element for two hours were made. After the application of the radium, some of the tumor was removed from the area immediately beneath where the radium had been, and at the same time tissue from the other side of the tumor, which was directly beneath the radium tube and never more than 1 to 1.5 cm. distant, was removed. Both of these pieces of tissue, together with the control tissue, were inoculated into a series of mice, and in some cases subsequent generations were carried on. After the first generation, the tumors arising from the tissue 1 cm. distant from the radium, showed an increase in size as compared with the control and with those grown from the tissue immediately under the radium. This stimulation effect often lasted through eight or nine generations of transplants.

From these experiments it is concluded: First, that the action of the gamma rays from radium in small doses upon tumor cells in vitro not only does not kill, but has a stimulating effect upon the cells, and that this effect persists through several series of generations of transplanted tumor.

Second, that the action of considerable quantities of radium upon tumor tissue in vivo, is effective only after long exposure, and then only upon the tissue directly beneath the radium. There is often a stimulating effect upon tissue 1 cm. distant. This points to the necessity for employing very large quantities of radium, 1 gm. or more of the element, if any lethal effect is to be obtained on cells lying more than 1 cm. from the active agent.

Discussion:

DR. PAPPENHEIMER: How does Dr. Prime explain the diminution in weight of the controls in the second and third generations? His charts seem to show quite marked variations in the rate of growth of the controls, as well as in the treated animals.

DR. EWING: I agree with Dr. Pappenheimer that in these experiments the controls are unsatisfactory. In one set the controls grew more than the treated specimens but in most of the others the controls hardly grew at all, so that any comparison with them seems unjustified. Would it not be possible to secure a tumor which will grow more uniformly?

It has frequently been stated that radium treatment may be followed by more rapid growth of the tumor. I have been unable to reach a conclusion whether in such cases the tumor cells have been stimulated, or the inflammatory reaction causes the tissues to swell, or the tumor merely goes on its natural course. The experimental stimulation of the mitotic process by radium has been proven only for very minute doses of radium. Clinically one observes that extensive carcinomas may continue to grow rapidly after inadequate treatment by radium, but it is difficult to say whether the growth has been stimulated. Recently I have seen a very extensive epithelioma of the lip which grew rapidly after radium treatment and it was feared that the growth had been stimulated. Later another patient with a similar tumor was admitted to the hospital, and on account of the previous experience it was not treated, but the same very rapid growth was observed. It is therefore of much importance that experiments such as those of Dr. Prime should be undertaken to test the matter, but even under experimental conditions it is not easy to reach a conclusion.

DR. LEVIN: I should like to cite an instance similar to Dr. Ewing's of a patient referred for treatment, suffering from a spindle cell sarcoma of the upper jaw. Radium therapy was advised but the patient did not return until four weeks later, when the tumor had increased six times in size. In this case obviously radium was not responsible for the increase in growth.

DR. WOOD: The discussion of this question shows clearly that the way to get an answer is not by studying individual cases but by carrying out experiments on such a large scale that accidental results can be eliminated. Regarding the variations in the rate of growth of control animals, it is known that tumors grow better in young than in old mice, and better in animals coming from one section of the country than in those from another section. In our experiments we have been careful to take these differences into account, but in spite of this care, differences in tumor growth do occur, as has been noted. Such variations make necessary the use of large numbers of animals. When many experiments are completed, it may even prove necessary to call in a statistician to help interpret the results. It is, however, obviously a matter of great importance to determine whether tumor growth can or can not be stimulated by the use of radium, and, to my mind, the question can be decided only by studying large series of animals, not by a consideration of individual cases.

DR. PRIME: There is no explanation for the variations in the rate of growth of tumors inoculated into a series of animals; but the point I wish to make is that if a hundred animals, all of about the same age and from the same breeder, are inoculated at the same time with the same amount of tumor tissue, the average weights of the tumors in groups of twenty-five or fifty animals will be very close. When inoculation is made from one of the tumors of this series into a second series, the average weights of the tumors may vary in different generations, but all the tumors in the same generation will be of about the same weight. The only way to obliterate these biological differences is to use very large series of animals, and thus show that such accidental errors can be eliminated while the variations following exposure to radium are constant.

THE RELATION BETWEEN VITAMINES AND TUMOR GROWTH

ISAAC LEVIN, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, M.D., Director)

It seems plausible to maintain, a priori, that there exists a certain relationship between the growth of a malignant tumor and conditions of nutrition within the organism of the host, and indeed there exists a certain amount of research which seems to corroborate it.

Ehrlich's[1] hypothesis of athrepsia gave the first impetus to this line of investigation. In accordance with this conception a tumor graft when inoculated into a new host must obtain there a certain specific food-stuff. When the supply of this so-called *x*-stuff in the organism is exhausted, either due to a deficiency of the diet necessary to furnish this specific *x*-stuff of food, or through a greater avidity of the normal body cells to the specific food-stuffs, then the graft fails to develop. The indirect experimental proofs of this hypothesis have no bearing on the present investigation and will not be discussed here, but a certain number of direct experimental studies will be analyzed.

Moreschi[2] in an attempt to furnish a direct experimental proof of the hypothesis of athrepsia studied the relationship between conditions of nutrition and the growth of an inoculable mouse sarcoma. He found that general underfeeding of the animal retards, to a certain extent, the development of a tumor graft. Similar results were reported by Haaland,[3] Stahr,[4] and Medigraceanu,[5] though their methods of research were not always as direct. Stahr for instance found that the difference in the takes of tumor grafts in different races of mice is due to variations in the feeding.

Rous[6] on the other hand obtained results which were not in complete accord with the previous investigations. He found that a quantity of food sufficient to sustain the life of the animals, but permitting of gradual emaciation, may influence the development of a tumor graft but does not impede the subsequent growth of a large tumor. Furthermore he found that different tumors react differently to the underfeeding of the animal. Thus, Flexner-Jobling carcinoma of the white rat does not seem to be influenced at all by low diet.

All the subsequent investigations were conducted, not on a generally restricted diet, but, on feeding with specialized foodstuffs. J. E. Sweet, E. P. C. White, and G. J. Saxon[7] took as the basis for their research the investigation of Mendel and Osborne.[8] The latter have devised a diet which while sustaining the life of young animals inhibits their growth. Sweet, White, and Saxon argued that, since body growth and tumor growth are analogous phenomena, Mendel-Osborne diet should impede the growth of a tumor graft. They experimented with a modification of Mendel-Osborne diet, and seemed to have obtained positive results, *i. e.,* the successful takes of tumor grafts was markedly reduced in animals fed with this special diet.

Van Alstyne and Beebe[9] also claim that qualitative differences in food influence the growth of malignant tumors. Their experiments consisted in addition of sugars to an otherwise normal full diet, and they found that this addition of sugar enhances the tumor growth while lack of sugar impedes it. Woglom[10] was unable to confirm these results. In his recent study on the subject Rous[11] attempted to approach more closely conditions as they obtain in human cancer. His aim was to observe whether a special diet would retard the appearance of a local recurrence and the formation of metastases after an incomplete surgical removal of the primary tumor. He employed for the experiments a Bashford mouse carcinoma, No. 63, Tyzzer's carcinoma of the walzing mouse, Flexner-Jobling carcinoma of the rat and spontaneous tumors. Sweet's modification of Mendel-Osborne diet was given to all the animals. The diet apparently checked the development of the recurrence in some of the tumors, but did not affect the Flexner-Jobling tumor.

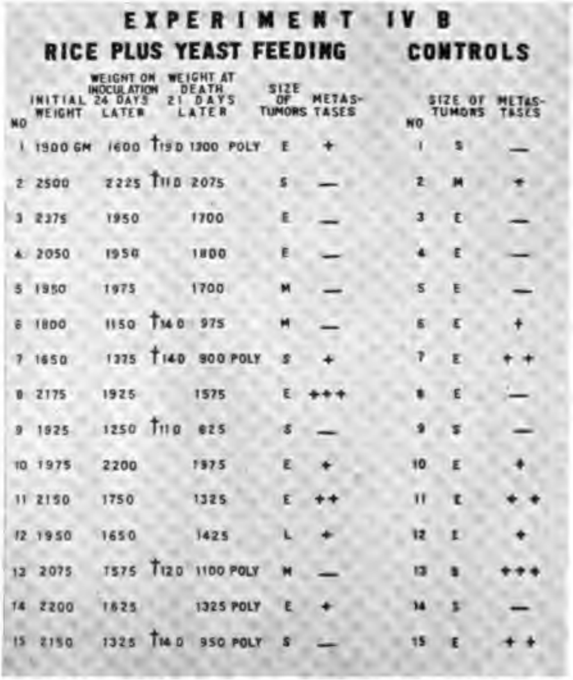
The results of all the investigations enumerated above do not appear to be sufficiently uniform or constant. On the other hand recent investigations on the etiology of beriberi and similar so-called deficiency diseases made very probable the existence in the food of a class of substances which C. Funk[12] named vitamines and which are of vital importance to the animal organism.

Vitamines are complex nitrogen containing crystalline substances, the exact chemical structure of which is not yet known. Deficiency diseases appear to be due to consumption of food which lacks the vitamines. Highly polished rice presents a food completely void of vitamines, while the rice-polishings contain all the vitamines of the rice. People living in the tropics and subsisting on polished rice as the only article of food suffer from beriberi and other deficiency diseases. An introduction into the organism of a small quantity of vitamine may cure a deficiency disease. An alcoholic extract of yeast is the most easily obtainable and the most active vitamine. Pigeons and chickens fed on polished rice emaciate, ultimately refuse to eat the rice and die of starvation. They also frequently develop a polyneuritis, which is an experimentally induced disease analogous to beriberi.

In view of the great importance of vitamine as a life-sustaining ingredient of food which moreover acts in minute quantities, it seemed plausible a priori, that of all the foodstuffs it should exercise the greatest influence on tumor growth, if the latter depends on conditions of nutrition at all. The writer, therefore, undertook to investigate the relation between vitamines and tumor growth. The sarcoma of the fowl described by Rous[13] is singularly well adapted for this line of research. The tumor is very malignant clinically, frequently forms metastases, and is readily inoculable in chickens. The latter animals on the other hand, as stated above, are easily influenced by changes in the vitamine contents of the food.

The experiments were conducted in the following way: 200 chickens divided in three groups were used for the experiments. The first group of animals were inoculated with the tumor simultaneously with the beginning of rice feeding. The second group of animals were fed with rice, and at the same time 20 c.c. of an emulsion of yeast extract in water (5 gm. of yeast extract in 20 c.c. of water) were introduced into the stomach of each chicken through a catheter every other day. The yeast extract was kindly prepared by P. A. Levene, of the Rockefeller Institute for Medical Research. Four weeks after the beginning of the rice and yeast feeding the animals were inoculated with the tumor. The object of this group of experiments was to enable the tumor inoculation to take place in animals already suffering from a deficiency of vitamine. The additional yeast feeding as conducted in this investigation does not cure the rice-fed animals completely, but prolongs their life. Instead of four weeks they could be kept alive nearly seven weeks. Nevertheless the animals became severely emaciated, and even a greater percentage of them developed polyneuritis than of the animals fed with rice only. This is probably due to the fact that the latter did not live long enough to develop the disease. The third group of animals was used as control to the other two. They were given normal food and were inoculated with the tumor usually on the same day and from the same source as the corresponding series of animals of the first or second group.

Chart I shows the result of an experiment on a series of fifteen animals of the second group and of fifteen controls. The first, second, and third vertical columns of the chart are self explanatory. The animals were killed twenty-one days after inoculation, since by that time the emaciation was extreme and they entirely refused the rice feeding. On the other hand the fowl sarcoma generally reaches its largest size at the end of three weeks. The cross, figure, and letter "D" to the left of the fourth column indicates the number of days of life after the inoculation, of animals which died before the end of the three weeks. The word "poly" to the right of the fourth column indicates an animal which developed polyneuritis. The letters in the fifth column indicate the size of the developed tumors. The tumors were always inoculated into the breast muscle of the fowls. Letter "E" indicates that the tumor infiltrated the *entire* breast muscle. Letter "L" means a *large* tumor the cut surface of which is not less than 10 cm. square. Letter "M" means a *medium* sized tumor the cut surface of which is not less than 5 cm. square. Letter "S" indicates a *small* tumor the cut surface of which is less than 5 cm. square. The dashes in the sixth column indicate the absence, and the crosses the presence, of metastases. The number of the crosses on one line shows the number of organs in which metastases were found in the animal.



EXPERIMENT IV B

RICE PLUS YEAST FEEDING						CONTROLS		
NO	INITIAL WEIGHT	WEIGHT ON INOCULATION 24 DAYS LATER	WEIGHT AT DEATH 21 DAYS LATER		SIZE OF TUMORS	METASTASES	NO	SIZE OF TUMORS	METASTASES
1	1900 GM	1600	†19 D 1300 POLY	E	+	1	S	—
2	2500	2225	†11 D 2075	S	—	2	M	+
3	2375	1950	1700	E	—	3	E	—
4	2050	1950	1800	E	—	4	E	—
5	1950	1975	1700	M	—	5	E	—
6	1800	1150	†14 D 975	M	—	6	E	+
7	1650	1375	†14 D 900 POLY	S	+	7	E	+ +
8	2175	1925	1575	E	+++	8	E	—
9	1925	1250	†11 D 825	S	—	9	S	—
10	1975	2200	1975	E	+	10	E	+
11	2150	1750	1325	E	++	11	E	+ +
12	1950	1650	1425	L	+	12	E	+
13	2075	1575	†12 D 1100 POLY	M	—	13	S	+++
14	2200	1625	1325 POLY	E	+	14	S	—
15	2150	1325	†14 D 950 POLY	S	—	15	E	+ +

Chart I

An analysis of the chart I shows that there is no distinct difference between the behavior of the tumor in the animals fed with rice plus yeast and in the controls. The number of animals with large tumors and metastases is somewhat greater in the control series than in the fed animals, but this difference is within the limit of normal differences within various series of animals taken at random. The same indefinite results were obtained in all the other series of experiments, indeed in some series the tumor appeared to grow better in the fed animals than in the controls. Extreme emaciation of the animal accompanied by the development of polyneuritis did not seem to prevent the growth of a large tumor and formation of metastases.

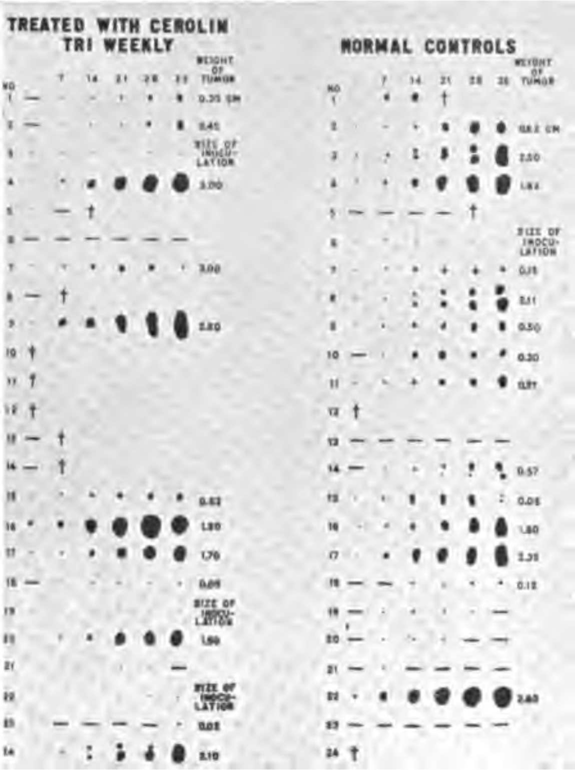


Chart II

Another mode of procedure to gauge the influence of vitamines on tumor growth employed in this investigation consisted in the introduction into the animal of additional quantities of the substance during the development of the tumor.

For these experiments a mouse carcinoma, No. 15 of the

Crocker Laboratory, was selected, because it grows comparatively slowly and gives approximately the same number of takes in the different series of animals. These characteristics of the tumor allow a sufficient time for the vitamines to influence the growth, and permit of a comparison between the number of takes and the size of the tumors of the treated animals and the controls.

Chart II presents a graphic description of one series of experiments: 48 mice were inoculated subcutaneously with 0.01 gm. of mouse carcinoma No. 15; 24 animals were injected three times a week subcutaneously with 3 mm. of a 10 per cent emulsion of cerolin in water. The latter is a commercial alcoholic extract of yeast. The other 24 animals were not treated and served as control. An analysis of the chart shows that the injection of cerolin did not enhance in any way the growth of the tumor. In fact the control series showed a greater number of animals with large tumors than the treated series.

Thus the growth of Rous's sarcoma of the fowl and of the carcinoma No. 15 of the mouse is not influenced by the variations in the amounts of vitamines taken in by the organism. While it is still feasible to presume a priori that variations in the conditions of nutrition of the animal may influence the growth of a tumor, even the finest experimental methods at our command at present cannot demonstrate such an influence. The metabolic differences between a normal body cell and a cancer cell are too finely adjusted to be detected by such comparatively crude methods as a quantitatively or qualitatively inefficient diet or even changes in the vitamine supply of the organism.

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SKELETAL METASTASES OF CARCINOMA

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A correct estimate of the frequency of skeletal metastases in malignant tumors is very difficult since the condition may exist for a long time without presenting any clinical symptoms or anatomical deformities. Moreover a routine autopsy in cases of malignant tumors as a rule does not include an examination of the bones unless there is present a distinct change in the configuration of a bone or a pathological fracture. Fraenkel[1] made a statement in a short society report on the subject of skeletal metastases, that the latter are found in about 20 per cent. of cases of carcinoma. Fischer-Defoy[2] in an analysis of 82 cases of carcinoma found skeletal metastases in 25.7 per cent. of the cases. Schmorl[3] reported that in a series of cases of carcinoma in his department, many bones were dissected and the marrow opened, even where there was no gross indication of any lesion. This investigation showed the presence of metastases in about 6 per cent. greater frequency than was found by Fraenkel and Fischer-Defoy.

An *x*-ray examination of the skeleton of cancer cases before or after death would easily reveal the presence of discrete metastatic nodules and obviate the necessity of sawing open all the bones of the body. A minute island of cancer cells in the center of the bone-marrow cannot be demonstrated on an *x*-ray plate, neither is it of any value for a statistical estimate of the frequency of skeletal metastases. On the other hand these minute nodules in the bone-marrow are usually found in the neighborhood of large metastases, and therefore for the purpose of a microscopical study of the early stages of metastasis formation, it is unnecessary to open all the bones at the autopsy and search for small metastases.

During the last three years the writer has had the opportunity to study 11 cases of skeletal metastases. The primary tumor was located in the breast in 7 cases, in the thyroid in 2 cases, and in the lung in 2 cases.

The investigation consisted in the first place of a Roentgenographic examination of the parts of the skeleton, in which the clinical symptoms indicated the possibility of the presence of a metastasis. In certain cases repeated *x*-ray examinations were made in order to study the changes in the development of the metastatic tumor. Furthermore, a microscopical study was undertaken of the skeletal metastasis of the cases in which an autopsy was obtained.

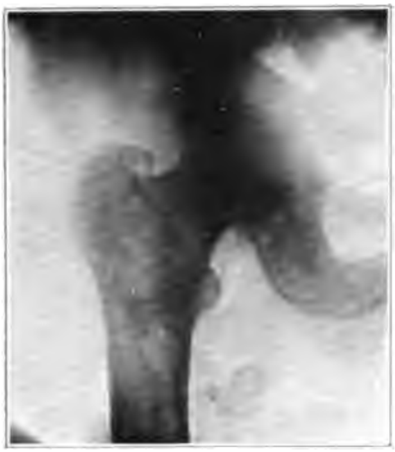
The following are brief histories of the cases.

CASE I. Mrs. A. S., age 54. Admitted to the hospital Nov. 20, 1913. Primary carcinoma of thyroid, metastases in the lungs and ribs. No satisfactory *x*-ray pictures were obtained. Died March 18, 1914. The metastases in the ribs served for pathological study.

CASE II. Mrs. M. G., age 43. Admitted to the hospital Dec. 3, 1913. An amputation of the breast had been done two years previous to the admission to the hospital. Patient died on Dec. 31, 1913. Autopsy showed metastases of carcinoma in the viscera and in the sternum. The metastasis in the sternum served for pathological study.

CASE III. Mrs. B. H., age 41. Admitted to the hospital Jan. 21, 1914, with an inoperable carcinoma of the right breast, painful spine and inability to walk. Clinical and *x*-ray study showed metastases in the eighth, ninth and tenth ribs, and in both femurs. Patient died May 20, 1914. Autopsy showed carcinoma of the breast and metastases in the axillary lymph glands, pleura, both trochanters and the eighth, ninth and tenth ribs. A microscopic study was made of the ribs and trochanters.

CASE IV. Mrs. T. S., age 39. Admitted to the hospital May 14, 1914, with a recurrence of a carcinoma of the right breast, which was amputated about six months previously. Clinical and x-ray study revealed metastases in the right femur and clavicle. Patient died June 17, 1915. Autopsy showed medullary carcinoma of the breast, metastases in the axillary, cervical, mediastinal, bronchial and retroperitoneal lymph glands and in the head of the right femur. The metastasis in the femur served for pathological study.



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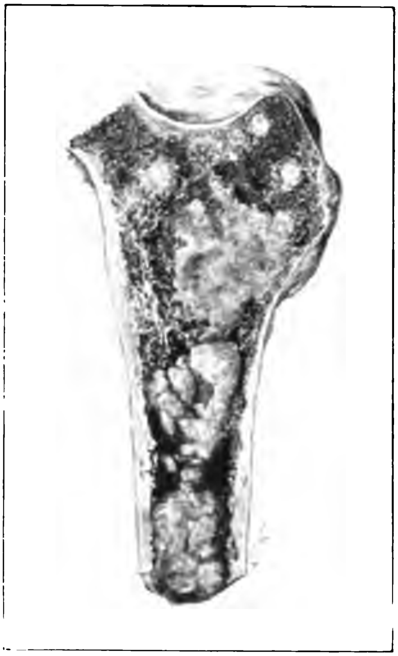

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FIG. 1. X-ray plate of the right femur of Case V, showing metastases.

FIG. 2. Photograph of the right femur of Case V, showing metastases in the bone-marrow.

CASE V. Mrs. F. E., age 56. Admitted to the hospital Nov. 10, 1914, with a recurrent carcinoma of the breast which was amputated in 1913. Three weeks after the operation, patient felt pain in the right thigh. Clinical and *x*-ray study revealed metastases in both thighs. One month after admission the patient sustained a fracture of the right femur at the level of the metastasis. The patient died on Jan. 1, 1916. Autopsy showed metastases in the liver, ribs and both femurs. Figs. 1 and 2 show an *x*-ray plate and a photograph of a sawed-open surface of the bone-marrow respectively. It is very striking that both bones showed the metastases practically in the same positions. The metastases in the femur and ribs served for pathological study.

CASE VI. Mrs. A. B., age 45. Admitted to the hospital Oct. 1, 1915, with a recurrent carcinoma of the left breast. The breast was amputated about six months previously. The patient complained of pains in the extremities which began half a year before the operation. An exhaustive *x*-ray study of the skeleton of the patient was undertaken and it showed the presence of metastases in practically every bone. The patient died on Jan. 22, 1916. No autopsy could be obtained.

CASE VII. Mrs. D. L., age 40. Admitted to the hospital Nov. 10, 1915, with a recurrent carcinoma of the right breast, which had been amputated six months previously. Two weeks after the operation the patient began to feel severe pain in the spine and became unable to walk. *X*-ray examination revealed metastases in the second and third lumbar vertebræ. At the time of writing the patient is still in the hospital. Subjectively the patient is better, she is able to walk about and a subsequent *x*-ray examination shows new bone formation in the region of the metastasis between the second and third lumbar vertebræ.

CASE VIII. Mrs. B. S., age 56. Admitted to the hospital July 6, 1915, with primary carcinoma of the lung. The patient died July 25, 1915. Autopsy showed adenocarcinoma of the lungs, metastases in pleura, liver, adrenal, ribs, vertebræ and in the lymph gland of the left axilla. The metastases in the ribs and vertebræ served for the pathological study.

Case IX. Mrs. R. R., age 43. Admitted to the hospital Jan. 16, 1916, with recurrent carcinoma of the breast which had been amputated about six months previously. About two months after the operation the patient began to suffer pain in the legs and back. Clinical and *x*-ray examination showed metastases in the eleventh and twelfth dorsal vertebræ, and the neck of the right femur. Patient died Feb. 13, 1916. Autopsy showed carcinoma of the breast with recurrence in wound and metastases in regional lymph glands, liver, pancreas, lungs, vertebræ and right femur. The metastases in the bones were studied microscopically.

Case X. Mrs. B. K., age 52. Admitted to the hospital Dec. 16, 1915. Died May 6, 1916. Autopsy showed carcinoma of the thyroid, metastases in the lungs and in the fourth lumbar vertebra. The latter was studied microscopically.

Case XI. Mr. K. R., age 62. Admitted to the hospital April 11, 1916, complaining of pain in the chest and coughing. His disease began seven months previous to admission. Clinical and *x*-ray examination showed a carcinoma of the lung. On July 29, 1916, there developed a tumor in the right clavicle. An *x*-ray examination showed destruction of the bone tissue and a pathological fracture.

The predilection of certain carcinomata to metastasize in the bones was ascribed to mechanical causes by von Recklinghausen,[4] who was the first to thoroughly investigate the subject. He maintained that the formation of metastases in the bones is due to the fact that the veins and capillaries of the bone-marrow have thin walls, and are not collapsible. These morphological peculiarities favor the accumulation of carcinoma cells within these blood vessels. The majority of pathologists side with von Recklinghausen in the opinion that the mechanical peculiarities are the main, if not the only factors in the causation of skeletal metastases. None the less this hypothesis is inadequate to explain all the phenomena connected with the formation of skeletal metastases. E. Kaufmann[5] gives a detailed account of a statistical investigation of the frequency of metastases in the bone. In 309 cases of carcinoma of the stomach bone metastases were formed in 2.5 per cent, in 159 cases of carcinoma of the uterus in 3.03 per cent, in 101 cases of carcinoma of the esophagus in 6.9 per cent, in 57 cases of carcinoma of the rectum in 10.5 per cent, in 63 cases of carcinoma of the breast in 52.3 per cent, and in 24 cases of carcinoma of the prostate in 66 per cent. The difference between 3.03 per cent of skeletal metastases in carcinoma of the uterus and 66 per cent in carcinoma of the prostate is very striking, and cannot be explained by mechanical differences. The topographic relationships between the uterus and the skeleton on one hand, and the prostate and the skeleton on the other are practically identical. So is the vascularisation of the two organs, and it is as easy for the cancer cells from the uterus as from the prostate to find lodgment in the bone marrow.

The opinion of Lubarsch[6] that the difference in the relative sizes of the cancer cells and of the minute blood vessels of an organ influence the formation of metastases, is also hardly tenable. The bone-marrow contains blood vessels of a sufficiently large caliber to admit the lodgment of a cancer cell of any size.

The only plausible explanation of this difference between tumors of certain organs seems to be that while both the cells of the uterine cancer and the carcinoma of the prostate find lodgment in the marrow, the latter cells find there a better soil for development, than do cells of the uterine cancer. A very striking instance indicating the existence of a peculiar affinity between the cancer cells and bone tissue was presented recently by Schmorl. He found in a series of cases of carcinoma, metastases, not only in the bones of the skeleton, but in bone tissue abnormally developed in the cartilage of the larynx and trachea, and in one case a metastasis developed in a bone plaque on the wall of the aorta. In the latter case no metastases were formed in any organ with the exception of the bones of the skeleton and of the bone tissue in the wall of the aorta. It is inconceivable that cancer cells should have been able to reach the small bits of bone tissue of the aortic plaque more easily than the parenchymatous organs.

Thus the probabilities are strongly in favor of the conception that tumor emboli are transported everywhere with the same ease and find lodgment in any part or organ of the body. The further growth, on the other hand, of the small transported group of cancer cells and its development into a large metastatic tumor depends upon a correct metabolic affinity between the cancer cells, on one hand, and the organ cells, the soil as it were, on the other.

The writer[7] has shown in a series of investigations, that the development and growth of inoculable malignant tumors of the lower animals depends upon a local interaction between tumor cells and the somatic cells of an organism. In certain instances the parenchymatous cells of a normal organ may inhibit the growth of the tumor, and this inhibition ceases when the parenchymatous cells become degenerated through a certain injury to the organ. On the other hand the writer[8] has shown that when the cell of a normal organ offers no adequate resistance to the growth of the tumor, then the proliferating tumor cells injure, mechanically or chemically, the former cells. This injury evidently precedes the death of the organ cells and their replacement by tumor cells.

Bone tissue, in view of its characteristic morphological differences from all other tissues, presents a very favorable object for the minute study of the changes taking place in the soil under the influence of tumor growth. In view of the further fact that such a study may help to elucidate not only the pathogenesis of metastasis formation but even the pathogenesis of cancer itself, a microscopical study was undertaken of the skeletal metastases of the cases reported here. The technique adopted in the present investigation was the following. Bone tissue containing the metastases was hardened in 5 per cent formalin and then decalcified in a 5 per cent aqueous solution of nitric acid. The latter solution was changed daily for four days. Further the tissue was washed for twenty-four hours in running water, hardened in 80 per cent and 95 per cent alcohol, imbedded in celloidin or paraffin and stained in hematoxylin-eosin, and by Van Gieson's method.

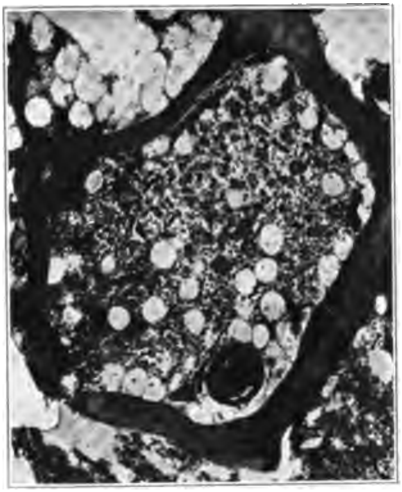

FIG. 3. Microphotograph of a bone-marrow space, showing a small island of cancer cells. (Low magnification.)

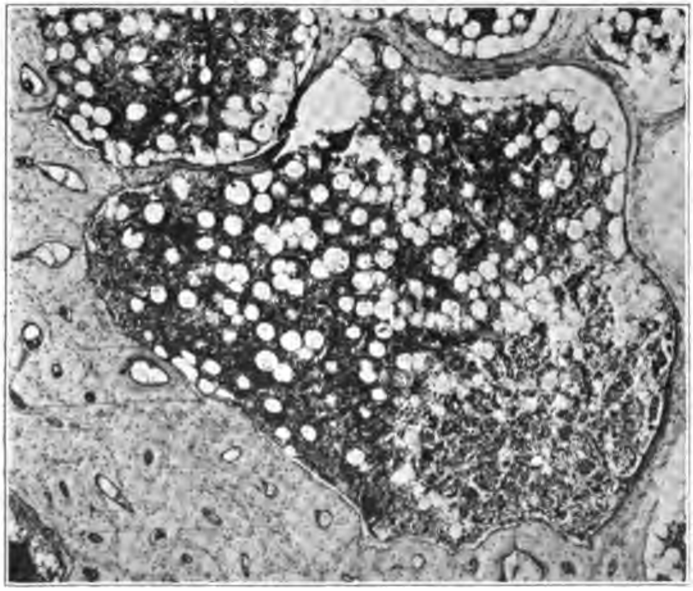

FIG. 4. Microphotograph of a bone-marrow space with a small nodule of carcinoma. (Low magnification.)

The microscopic study of the material brought out several points of importance.

The metastasis begins its development within the marrow, and when the group of cancer cells is small, the surrounding bone-marrow appears quite normal. Figs. 3 and 4 present microphotographs of two such small cancer nodules within a bone-marrow space. Von Recklinghausen claims that the development of the metastasis is preceded by a hyperæmia and hæmostasis, due to obstruction of the capillaries by the tumor emboli. In the specimens studied by the writer, hæmorrhages and hyperæmia were noted frequently around large tumor masses, in the femur for instance (see Fig. 2), but not around minute metastatic nodules. In the latter instance as stated above no morphological abnormality could be found in the bone-marrow.

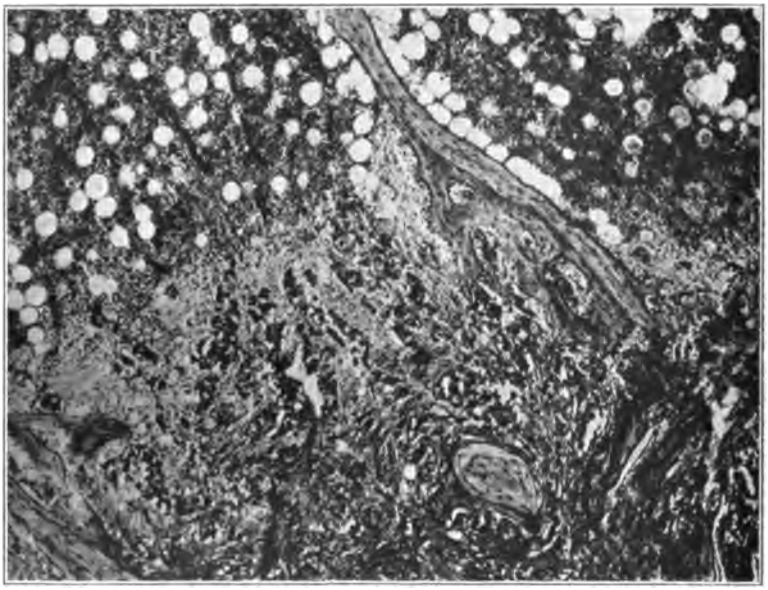

FIG. 5. Microphotograph, showing abundant new bone formation in the vicinity of an old bridge of compact bone tissue.

As the tumor nodule increases in size it approaches and invades the compact osseous tissue or the compact osseous partitions of the cancellated bone. Then, there begin to appear the characteristic changes in the bone tissue. It is generally accepted that there are two classes of skeletal metastases of carcinoma: *osteoplastic,* in which extensive new bone formation takes place around the metastatic tumor; and *osteoporotic,* in which the changes in the normal tissue surrounding the metastasis consist in extensive destruction of the compact bone. These two distinct conditions can be easily differentiated on the gross inspection of skeletal metastases. However, the microscopic study of the cases in the present investigation has shown that both conditions are usually present side by side. The differences in the gross appearance are due to the fact that in one case *osteosclerosis* or new bone formation predominates, while in another *osteoporosis* or the destruction of the old bone is mostly in evidence. Fig. 5 shows an extensive new bone formation, while Fig. 6 presents only destruction of the old bone, and both speci-

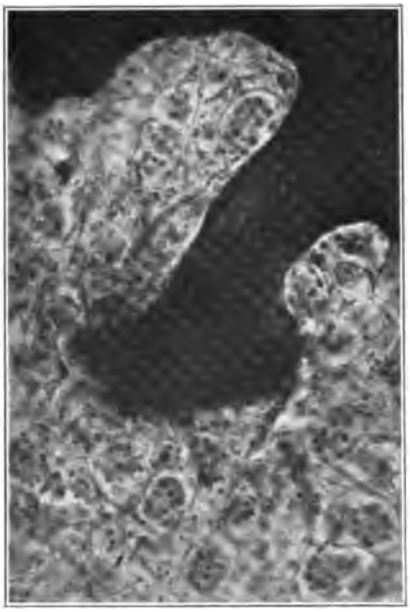

FIG. 6. Microphotograph, showing two bone lacunæ filled with carcinoma cells. (Low magnification.)

mens were obtained from different regions of the same metastatic tumor.

The mechanism of the bone destruction in metastases of carcinoma differs from the one observed in inflammatory osteoporosis. Von Recklinghausen first made the observation that the large polynuclear osteoclasts, which destroy the bone in osteoporosis, are very seldom found in the lacunæ of the bone, surrounding a growing metastasis of carcinoma. This fact was confirmed by most of the subsequent investigators. In view of the absence of the large osteoclasts von Recklinghausen presumed that there takes place in this bone a softening by the removal of its inorganic salts and a subsequent absorption without the aid of any cells, a condition similar to the one found in osteomalacia. Apolant,[9] Erbslöh,[10] Askanazy[11] are also of the opinion that osteoporosis in skeletal carcinoma may take place without the aid of special cells. On the other hand, Wolff[12] and Goetsch[13] believe the cancer cells act as osteoclasts and destroy the compact bone and Axhausen[14] maintains that the small elongated mononuclear connective tissue cells frequently found close to the walls of the lacunæ are special osteoclasts derived from the cancer stroma. In the specimens studied by the writer both carcinoma cells and the small connective-tissue stroma cells were found in immediate apposition to the walls of the lacunæ. The carcinoma cells were so frequently the only cellular elements within the lacunæ of the bone, that there cannot be any doubt but that carcinoma cells do act as direct osteoclasts. Fig. 6 shows two lacunæ filled with carcinoma cells. The impression gained by the writer is that the small stroma cells only subsequently invade between the bone and the carcinoma cells. But even if the possibility be admitted that occasionally the stroma cells may act as osteoclasts, it seems quite apparent that the tumor itself by the aid of its formed elements first destroys the bone, and then grows by occupying the space so formed.

While the mechanism of osteoporosis in skeletal metastases is similar to the mechanism of the destruction of any normal tissue surrounding a growing malignant tumor, the osteosclerosis or the extensive formation of new bone tissue is characteristic only of skeletal metastases.

Von Recklinghausen claims that this extensive new bone formation is due to the hyperæmia described by him and mentioned above. Courvoisier[15] and Kaufmann maintain that a metastasis in the bone is always surrounded by a zone of inflammation, and that this latter causes the osteosclerosis. Askanazy and Assman think that the metastasis at first produces an osteoporosis which is followed by a bone necrosis. The necrotic bone acting as a foreign body causes a new bone formation. Wolff, Courvoisier and Kaufmann are of the opinion that carcinoma cells may act directly as osteoblasts and form new bone tissue. Fig. 7 presents a picture very frequently observed by the writer. It shows collagen fibrils formed in abundance from the old bone. These fibrils gradually unite in thick bundles and subsequently form new bone tissue. The latter is clearly formed from the constituent parts of the old bone tissue. On the other hand, as stated above, the writer did not observe any hyperæmia, inflammation or any other abnormality of the bone-marrow at the beginning of the development of the metastases. Neither did

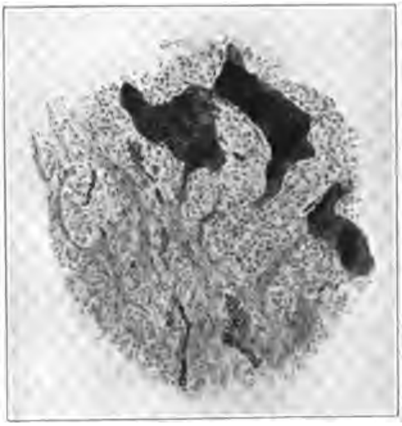

FIG. 7. Drawing showing collagen fibrils emerging from the old bone and uniting with other fibrils.

the writer find any necrosis of the bone in any of the specimens examined. It must be concluded in agreement with Axhausen's conceptions that some unknown chemical irritant emanating from the carcinoma cells acts on the old bone tissue and stimulates its proliferation.

Thus two processes always take place side by side in skeletal metastases. On the one hand the tumor destroys the normal bone tissue, on the other hand the remaining osseous tissue proliferates and creates new bone. The latter is quite probably an attempt at self-defense. The new bone tissue may compress and destroy the cancer cells. Case V shows such a condition. A pathological fracture occurred in the right femur, which subsequently became firm and clinically seemed healed. Later a second fracture took place below the first. Fig. 8 shows an *x*-ray plate of the bone. Such temporary healing of a pathological fracture within a skeletal metastasis has been described on several occasions.

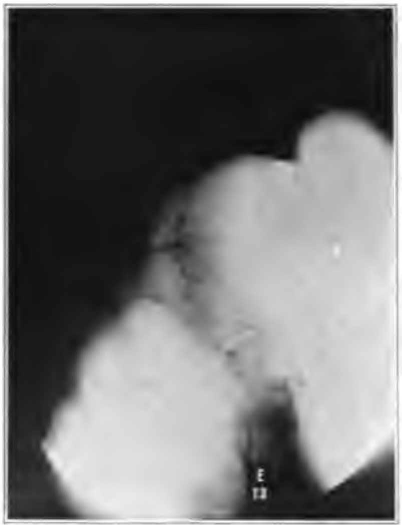

FIG. 8. *X*-ray plate of the head of the left femur of Case V, showing a double fracture.

The minute microscopic study of skeletal metastases gives the clearest evidence of the interaction which always takes place between the normal organ tissue and the cancer cells during the beginning of the development of a metastatic tumor from a transported cancer embolus. Upon the result of this interaction depends the success of metastasis formation.

The subject may be of practical importance. New bone formation begins as stated above by a new formation of connective tissue (collagen fibrils). The formation of connective tissue is always enhanced by radiotherapy and it is thus possible that radiotherapy may aid in the attempt at self-defense mentioned bove. The writer will consider this subject extensively in another place.

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Discussion:

Dr. Wood: It seems possible that the small size of the cells characteristic of many prostatic carcinomata may account for the high percentage of bone metastases in tumors of the prostate. In cases of breast carcinoma with large cells, nodular or skeletal metastases are rarely seen; whereas in scirrhous growths with small cells there may be early and extensive secondary growths. It seems reasonable to assume that the small cells can more readily enter the lymph channels.

GAUCHER SPLENOMEGALY IN A CHILD

FRANK A. EVANS, M.D.

(From the Pathological Laboratory of the Presbyterian Hospital, New York)

This paper is a preliminary report on a case of primary splenomegaly of the Gaucher type, associated with symptoms pointing to lesions of the central nervous system. A complete report will be made later in collaboration with Dr. Tilney, who is studying the nervous lesions.

The patient, a boy, four years and eleven months old, son of a well-to-do Austrian Jewish family of Brooklyn, is the second of three children. The family history is entirely negative. On physical examination the father and mother showed nothing of interest in this connection. The other two children, girls aged nine and three years, have never been ill and physical examination revealed nothing of pathological significance, notably, no anemia and no enlargement of the spleen. The mother has had no miscarriages. All three of the mother's pregnancies were uneventful.

The birth of this patient was instrumental. He was breast fed for about a year and thrived normally during this time. After this he showed signs of indisposition and when eighteen months old had an attack of bronchopneumonia. As a child he had many hard falls and his mother noticed that he always bled easily from the nose and mouth. In one of these falls the patient injured his back and subsequently was treated in a hospital as a case of Pott's disease. At this time, when he was two years old, his physicians observed that his spleen was enlarged. When three and one half years old the left hand and foot began to twitch and he fell frequently as before, but now always to the right side. Later both sides and the face became involved in these twitching movements. One year ago a bilateral decompression was done at Mt. Sinai Hospital, after the first of which the patient was temporarily relieved of the symptoms. When this twitching recurred, however, it was as severe as before, and was subsequently associated with clonic spasms and attacks of grand mal. While at Mt. Sinai Hospital an enlargement of the liver and spleen was again noted; and here also a Wassermann test on blood and spinal fluid was negative. During the next year the nervous symptoms became worse and the day before his death the patient was sent into the Presbyterian Hospital. From the time he came into the hospital his temperature began to rise rapidly, reaching 109° F. the day of his death, which occurred soon after admission. An autopsy was performed three and a half hours post mortem.

AUTOPSY REPORT

The body was fairly well nourished and developed for a boy of five years. The skin and mucous membranes were very pale but showed no rashes or petechiæ and, except for some livor mortis over the back and sacrum, no areas of discoloration. A bloody discharge from the nose and mouth was noted. There was no superficial glandular enlargement but the inguinal glands were palpable, although soft and discrete. There were several ragged scars over the forehead and the scar of a decompression in each temporal region. The pupils were round and equal, the conjunctivæ and scleræ clear.

On opening the chest a thymus extending 6 cm. below the episternal notch and weighing 12 gm. was exposed. The lungs crepitated throughout but on section showed small red patches in the region of the bronchi. The bronchi were injected and full of bloody mucus. The glands at the hilum were slightly enlarged and pigmented. The heart was normal except for small red patches in the epicardium over the body of the left ventricle, the remains of old petechial hæmorrhages.

The liver was large, weighed 600 gm., and extended 1.5 cm. below the costal margin in the right mid-clavicular line. It was soft and flabby and flattened out on the hand. On section the parenchyma bulged above the line of the cut surface. The color was a pale yellowish-brown. The lobulation was very indistinct.

The spleen weighed 350 gm. It was reddish in color and in one area over the posterior surface showed some fibrinous adhesions. The normal shape of the spleen was maintained. On section the parenchyma was firm, and little material could be scraped from the surface. It was predominantly a pale red color but was mottled with darker red pin-point areas. The trabeculæ were not prominent. The usual appearance of the Malpighian corpuscles could not be made out.

The intestines showed hyperplasia of the lymphoid tissue in the terminal portion of the ileum.

The mesenteric and retroperitoneal lymph nodes were enlarged, varying in size from those just palpable to others one cm. or more in diameter. They were discrete and soft. On section they were a pinkish-gray color of even distribution, except in a few where the reddish tinge was denser in the center.

The bone marrow from the upper third of the femur was moist and dark red in color.

Further than these findings, except for changes in the central nervous system which will be included in the subsequent report, there were no pathological changes.

MICROSCOPICAL EXAMINATION

The lungs showed an intense bronchitis with hæmorrhage in the lung tissue surrounding the bronchioles.

The spleen presented a remarkable picture. The parenchyma was made up of small groups of large cells, surrounded by a thin connective-elastic tissue capsule, and separated from each other by small amounts of normal looking splenic pulp. Some of the groups of cells were densely packed, but in other places they were not so crowded and lay free, together with a few red blood cells, in what could in these places be seen to be venous sinuses. Some of these sinuses appeared to be lined with swollen endothelial cells and others by cells similar to those free in the lumen. The supporting tissue around these sinuses and groups of cells was scanty but everywhere present. The pulp between the distended sinuses was not abundant but appeared to be made up of the elements normally encountered. The Malpighian corpuscles were widely separated but normal in size, and at the center of each were many of the same large cells that distended the venous sinuses. The usual lymphocytes were somewhat scanty in the center and many of them were fragmented. Surrounding each Malpighian corpuscle was a small zone of hæmorrhage, which gave to the organ its mottled gross appearance. The trabeculæ were about as usually seen. The lymph glands showed many of the same type of large cells that distended the venous sinuses of the spleen. These were grouped in long cords or small masses for the most part, but some were isolated. The larger groups of these cells were commonly surrounded by a delicate connective and elastic tissue strand, and the long columns were frequently bounded on each side by blood vessels. Lymphoid cells were necessarily reduced in number, but still very numerous. The germinal centers contained many of these large cells and, as in the Malpighian corpuscles of the spleen, the lymphocytes were

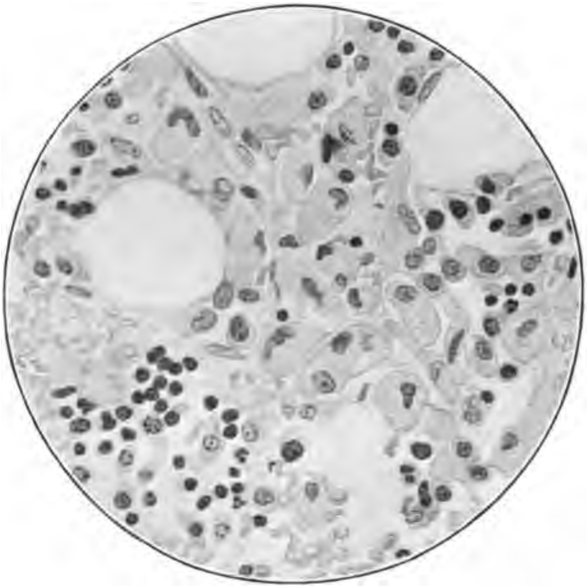

FIG. 1. Bone marrow, showing large cells, with reticulated protoplasm.

often fragmented. The mediastinal lymph glands and those at the hilum of the lung showed the same cells as were found in the retroperitoneal and mesenteric glands. It was interesting to note that none of these large cells in the mediastinal glands contained anthracotic pigment, although other cells in the same section did.

The lymphoid tissue in the terminal portion of the ileum showed changes similar to those described for the lymph glands. The bone marrow showed, richly scattered throughout its substance, many of these pathological cells, singly and in groups. Although many of them were lying on strands of reticular tissue, many were not, and here as elsewhere the relation of these cells to any specific element was not clear.

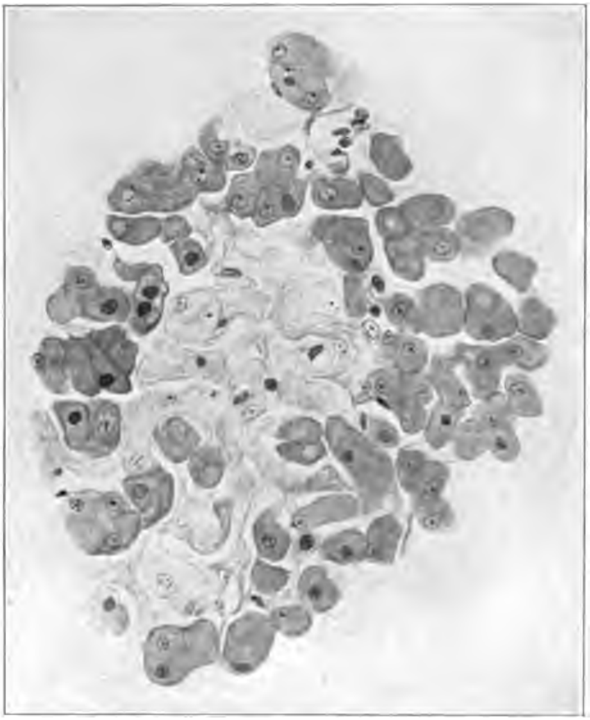

FIG. 2. Liver, showing a group of large cells between the liver cells.

The liver presented a lesion difficult of interpretation with the ordinary stains thus far used. The normal-looking liver cells were swollen. Beside these, scattered everywhere throughout the section were smaller, shrunken-looking cells, more faintly staining than those identified as liver cells. They might not be, however, recognized as the same pathological cell that was present in the spleen, but it seems probable that this change was part of the same process, not so frankly manifested here as in the spleen and blood-forming organs.

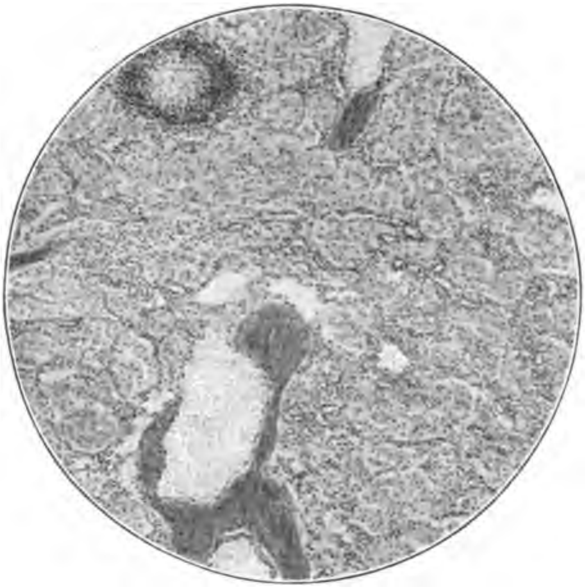

FIG. 3. Spleen.

None of the organs involved in the pathological process contained any iron pigment. The other organs of the body were examined with negative results and will not be mentioned by name, except to say that the enlarged thymus did not show any pathological cells.

The pathological cells in all of the organs except the liver were essentially the same. They were large, varying from 20 to 40 microns in diameter, often round, but more commonly oval or elongated. Sometimes they were drawn out into long strands. The nucleus was small (7 microns) and densely staining in most cells, but in a few was larger and clearer. It was always eccentrically placed. The protoplasm was abundant, faintly staining, and contained no vacuoles. There was often, however, an irregular area, usually on one side of the nucleus and extending up to it which stained very faintly or not at all. The protoplasm contained delicate strands running in the long axis of the oval or elongated cells. In those that were round these strands were difficult to trace but seemed to take no particular course. Where the cells were crowded together in long sheets and were drawn out, this longitudinal striation of their protoplasm gave a characteristic appearance to the section. Occasionally these cells were crowded together into what appeared to be a syncytial sheet. The formation of giant cells was very rare. These cells contained no lipoid that could be demonstrated by osmic acid, Sudan III, Scharlach R, or the myelin sheath stain of Wright.

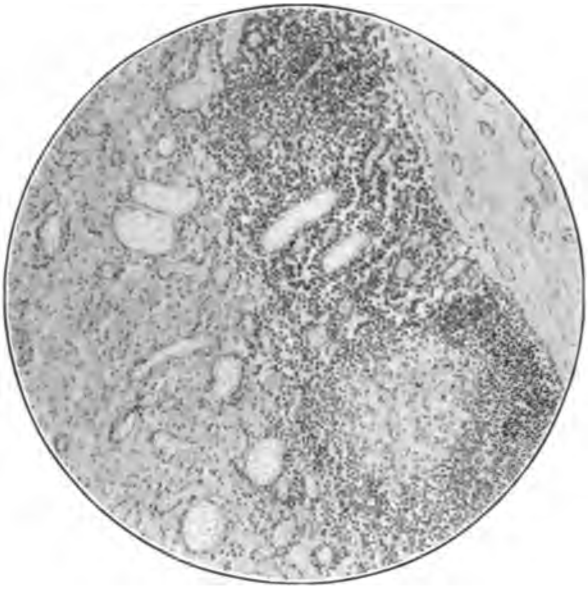

FIG. 4. Lymph Gland.

They did not contain an oxydase ferment when tested with the indophenol blue reaction, and no mitochondria could be seen in them after staining with Heidenhain's hematoxylin or with acid fuchsin and methyl green after the method of Bensley.[1] As to the direct relation of these cells to other elements, little can be said with certainty. Van Gieson and Mallory connective tissue, and Weigert's elastic tissue stains showed that, in all the organs involved in this process, these pathological cells were often in close relation to reticular elements, and, where densely packed in the sinuses of the spleen, were lying directly on the reticulum. In some of the sinuses, where not so densely packed, they were separated from the reticular tissue elements by a layer of swollen cells that strongly suggested endothelium.

INTERPRETATION OF THE AUTOPSY FINDINGS

In view of the history and pathological findings we believe that this is an example of primary splenomegaly of the Gaucher type. It differed from the typical picture as established by Bovaird,[2] Risel[3] and Mandlebaum,[4] in several respects. No other members of the family are at present suffering from a similar affection; there were no discolorations of the skin or lesions of the conjunctivæ, and the liver was not so extensively or frankly involved in the process as in the classical type. The case is of further interest in that it was associated with symptoms pointing to a lesion of the central nervous system. As yet we have not determined whether this was closely associated with, or entirely independent of the Gaucher's disease.[1]

It is noteworthy that the same question as that of the origin and relationship of these pathological cells, arises also in regard to the so-called reticulo-endothelial cells of Goldman,[5] Evans,[6] Kiyono,[7] and others, which are specifically stained intra vitam by pyrrol blue, trypan blue, lithium carmine, etc. These cells have practically the same distribution in the body as the pathological cells in Gaucher's disease. It would be of interest in cases of Gaucher's disease to study carefully the milky patches of the omentum which are made up almost entirely of these vitally staining cells. In this case, unfortunately, no sections were taken from the milky patches, but gross examination of the omentum showed no abnormality. On the other hand, in this case the mediastinal lymph glands showed the vitally staining cells loaded with anthracotic pigment side by side with the pathological cells, none of which contained any pigment. Until Gaucher's disease is reproduced in animals capable of being vitally stained, or until a case of typhoid fever superadded to Gaucher's disease becomes available, no positive statement can be made about the identity of these pathological cells with the normal vitally-staining cells of the body. However, a study of this case has led me to the belief that the pathological cells are not of endothelial origin exclusively, or of reticular origin, or of lymphoid character arising in the germinal centers of the lymph glands and Malpighian corpuscles alone; but that they are identical with the vitally-staining reticulo-endothelial cells, and that knowledge acquired as to the latter cells will apply to the pathological cells of Gaucher's splenomegaly. The agent exciting the great proliferation of these cells in Gaucher's disease is not known, but the reticulo-endothelial cells proliferate moderately under certain known circumstances,[8] and one author[9] gets a proliferation of similar cells by the injection of lipoids. I realize that this statement will meet opposition, and admit that a careful differentiation between the large cells of lipoidemia[9,10,11] and those of Gaucher's disease must be made. But I feel that careful metabolic studies on cases of Gaucher's disease might be of value in throwing light, not only on the etiology of this disease, but also on the physiological activity of that large class of tissue cells, the histogenous macrophages.

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[1] If these pathological cells of Gaucher's disease can be shown to be the same as the vitally staining cells, the association of nervous lesions with this disease might be expected. McCurdy and Evans (*Berl. klin. Woch.*, 1912, xlix, 1695) have shown that the vitally-staining histogenous macrophages can, and under some conditions do, proliferate in the central nervous system.

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Discussion:

Dr. F. S. Mandlebaum: This case of Dr. Evans interests me particularly because the liver apparently showed no involvement. If this be a true case of Gaucher's disease, and Dr. Evans's presentation leads me to believe that it is, it would be the first case reported without liver involvement. It is often difficult to demonstrate the large cells in liver sections and Dr. Evans should not despair of finding them. The phosphomolybdic acid hematoxylin stain of Mallory will often bring them out when other methods fail. It is interesting that this child was the only affected member of the family, but I know other instances where this is also true. The absence of pigmentation of the skin as well as the absence of the conjunctival lesion might be expected in so young a subject.

It should be emphasized that in true Gaucher's disease no lipoids are found in the cells by microchemical methods. The peculiar substance which is present in the large cells is not soluble in ether or alcohol, but is rendered more prominent by these reagents. A careful study of the morphology of the cell, however, is sufficient to distinguish Gaucher's disease from the lipoidemia cases. The cells in the cases of diabetes associated with lipoidemia, as well as the cells in Anitschkow's experimental animals fed on cholesterin, have a more or less rounded contour, and the vacuoles from which lipoids may be extracted are always round. In Gaucher's disease, on the other hand, the cells show a peculiar streaked or wrinkled appearance of the cytoplasm, and round vacuoles are absent. We have stated definitely, therefore, that the diagnosis of Gaucher's disease may be excluded in any case which bears a resemblance histologically to Gaucher's disease and in which lipoids can be demonstrated in the large cells by microchemical methods.

We agree with Dr. Evans that the large cells are derived from the reticulo-endothelial apparatus of the hemopoietic organs, and that in all probability they are identical with the cells involved in lipoidemia, in vital staining, and in Anitschkow's cholesterin fed animals. The substance in the cells, however, differs in each instance and depends upon the underlying etiological factors. The results of our chemical analyses lead us to believe that in Gaucher's disease some metabolic disturbance with the production of a complex protein substance, probably in combination with the phosphatids, must be considered.

DR. LAMBERT: I have studied sections from Dr. Evans's case and I do not think he intended to say that the liver was not affected. The liver was indeed very much altered. There were queer, pale areas in which the cells did not resemble normal liver cells, but they were different also from the large cells of the spleen and lymph nodes and we hesitated to interpret them as Gaucher cells.

A CASE OF LYMPHOSARCOMA OF THE SIGMOID

A. L. GOODMAN, M.D.

The patient, a child of four years, was admitted to the German Hospital, Feb. 24, 1916, complaining of chronic constipation and bleeding from the rectum, accompanied with extreme pallor and marked weakness. The child had been somewhat constipated for about four months, and more recently had had severe abdominal cramps before defecation. For three weeks there had been frequent attacks of tenesmus, accompanied by the passage of pure blood—at one time as much as half a pint. When the severe symptoms began the mother noticed a hard lump in the child's abdomen, which seemed to change its position from day to day and to grow gradually larger.

On admission the child did not seem acutely ill. She was extremely pale but fairly well nourished. The cervical, axillary and inguinal nodes were palpable and the tonsils markedly hypertrophied. There were signs of mitral insufficiency and hypertrophy of the heart. The abdomen was scaphoid in type.

On inspection a tumor was seen occupying the right umbilical and inguinal region. On palpation this could be felt as a hard, irregular, nodular mass which seemed very superficial. It lay parallel to Poupart's ligament and measured 13.5 cm. in length and 5.5 cm. in its broadest diameter, being twice as broad above as below. It could be displaced beyond the median line and above the umbilicus and moved with respiration. The tumor was roughly sausage-shaped. It was rather doughy to the touch and not tender. There was no tenderness or rigidity of the abdominal wall.

Physical examination showed nothing else of importance. The Wassermann and von Pirquet tests were negative.

The blood count was as follows: Hemoglobin 55 per cent.; white blood cells 1,160; polymorphonuclears 62 per cent.; lymphocytes 38 per cent. The blood pressure was 110. The urine contained a faint trace of albumen, many pus cells, a few red blood cells, but no casts. Examination of the feces showed pure blood, blood clots; scant fecal material and no ova.

On admission to the hospital the abdominal tumor lay in the right iliac region. With care it could be moved from one side of the abdomen to the other. Two days later, February 26, the tumor lay in a transverse position across the abdomen, corresponding to the transverse colon. On February 28, it had moved across to the left side of the abdomen just below the splenic flexure of the colon.

An *x*-ray examination revealed a distinct obstruction in the large intestine, which apparently was in the distal portion of the transverse colon.

The differential diagnosis was interesting. The history was of a rather acute illness of three weeks' duration. At the onset she was seized with sudden abdominal pain, so severe that she lay on the floor and screamed with pain. Shortly afterward the child passed blood from the rectum. The blood was dark in color and clotted, which would indicate an origin high up in the intestine. The sudden appearance of the tumor and its sausage-shaped character, would lead one to consider the possibility of an intussusception of the intestine. Against the diagnosis were the painlessness of the tumor, the absence of vomiting, and the duration of the swelling as well as the general glandular enlargement which spoke more in favor of a new growth. The negative von Pirquet and absence of signs of a general tuberculosis, excluded the possibility of a tuberculous condition in the omentum. The constant loss of blood, the increasing pallor, and the gradual increase in size of the swelling made operative interference seem advisable.

A laparotomy was performed on February 28. A tumor of the sigmoid was found, about 6 inches in length, and three inches in width, with metastatic involvement of lymph nodes in the mesentery. The mass was removed and the two ends of the gut were joined together by means of a Murphy button. On March 4 the child died with symptoms of general asthenia.

The pathologist's report is as follows:

Gross Examination: The specimen consists of a portion of the sigmoid with an incorporated tumor mass. The entire specimen measures 33 cm. in length. At either end is a segment of approximately normal gut, one measuring 13 cm. in length, the other about 1 cm. The remainder of the intestinal wall is enormously, but somewhat irregularly thickened, so that it forms an ovoid tumor mass about 6.5 cm. in diameter at its widest part. Through this mass the lumen of the intestine runs eccentrically. The thickening of the walls extends unevenly around the whole circumference, in the thinnest portions measuring about 1 cm.; in the thickest, about 3 cm. On section through the growth, the mucosa of the intestine appears to be replaced by a white nodular tumor mass which in one area is extensively ulcerated. The cut surface of the gut wall is of chalky white color resembling lymphoid tissue. The outer surface is irregularly nodular and rather soft. In the mesentery are found some enlarged lymph nodes.

Microscopical Examination: A section through the tumor shows the original structure of the intestinal wall to be practically obliterated by a diffuse sarcomatous infiltration, composed of closely packed small round cells about the size of lymphocytes. Only in a few regions are found traces of the smooth muscle and of the original mucous membrane. Externally there is a thin capsule of fibrous tissue, evidently peritoneum. but this also shows some infiltration with tumor cells in most regions. The blood vessels are very scanty and consist for the most part of thin-walled capillaries unsupported by connective tissue. Nevertheless, there are no marked areas of necrosis in the sections examined. At its margins the tumor invades the stroma of the mucous membranes and appears gradually to merge with the normal lymphoid tissue of the mucosa. Mitotic figures are exceedingly abundant.

Anatomical Diagnosis: Lymphosarcoma of the large intestine.

One finding seemed to explain the passing of dark clotted blood in this case. In the center of the tumor was a small cavity with three deep ulcerations in its wall from which bleeding took place. The blood apparently accumulated in this cavity and clotted, and as the opening from the lower end of the tumor mass into the gut was so small that one could hardly force the little finger through it, these clots may have remained in the cavity for some time before being passed. Such a possibility should be taken into account when considering the significance of clotted blood in the stools.

In reviewing the literature of intestinal lymphosarcoma it is interesting to note that in most of the cases reported, the onset of the symptoms was acute. The reason for this is apparently that with the growth of the tumor the lumen of the gut is gradually restricted until it becomes so small that it is impossible for the fecal matter to pass. This obstruction gives rise to symptoms which simulate acute or chronic intussusception of the intestine, from which condition it must always be differentiated.

These cases, while not extremely rare, are infrequent enough to warrant an individual report.

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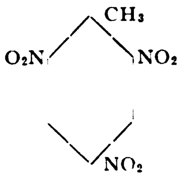
TRI-NITRO-TOLUENE POISONING

H. S. MARTLAND, M.D.

(From the Pathological Laboratory of the City Hospital, Newark, N. J.)

Tri-nitro-toluene has the following graphic formula:



It is known in the munition industry as TNT, and is the high explosive of the present European War.

It has from fifteen to twenty times the explosive strength of nitro-glycerine. To explode TNT, it is necessary to bring it up to a certain heat and then produce concussion. Heat alone causes it to burn like gum-camphor, concussion alone will not explode it. A shell filled with TNT is usually exploded by placing in the nose of the shell a detonator containing another high explosive such as fulminate of mercury.

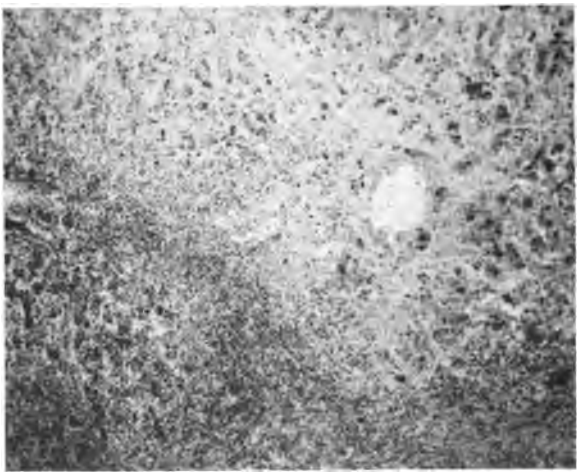


FIG. 1. Tri-nitro-toluene Poisoning. Section of liver showing extensive and diffuse necrosis and fatty degeneration, with perilobular lymphocytic and polynuclear infiltration.

In the manufacture of TNT there is little danger of industrial poisoning, since the procedure takes place in closed retorts, etc., with, consequently, no exposure of the workmen to the fumes. In the filling of high explosive shells it is necessary to heat the flaked TNT above its melting point, to about 94° C., and after letting it cool slightly, to pour the molten resin-like substance into the steel shell and then allow it to solidify. It is during these procedures that the workmen are exposed to the heavy fumes emitted by the hot TNT. It is afterwards necessary to chip and scrape off the solidified explosive which has collected around the top of the shell. An accurate core must also be drilled into the solidified TNT to make room for the detonator. During these processes, especially if proper protective machinery is not in use, the workmen are exposed to the yellow dust.

It is very probable that TNT poisoning has been known in Germany for some time. Reports of it however have not been made public. There has been little about it in the French or English literature until quite recently. In 1915, in England, a committee was appointed under the chairmanship of Sir George Newman to make a report to the Ministry of Munitions on industrial diseases occurring in munition factories and workshops. In a part of their report, Memorandum VIII, the symptoms of TNT poisoning are described and measures for minimizing the risks are outlined.

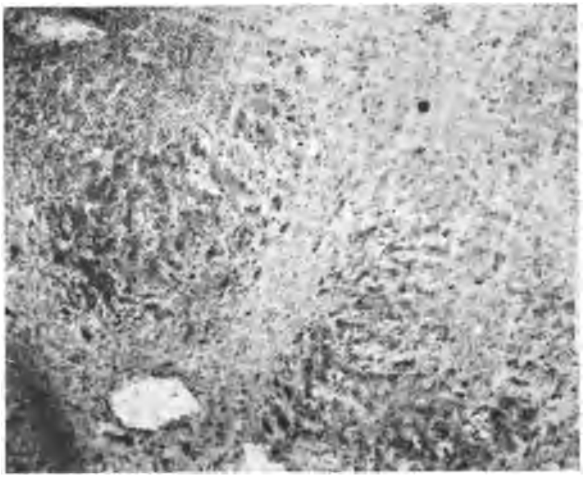


FIG. 2. Tri-nitro-toluene Poisoning. Section of liver showing extensive necrosis with bile-duct proliferation around periphery of lobule.

In the early part of 1916, Spilsbury (1) demonstrated before the Section of Pathology of the Royal Society of Medicine four livers presenting the following types of toxic hepatitis:

1. *Tetra-chlor-ethane Poisoning.*—Tetra-chlor-ethane is used to dissolve acetic cellulose in preparing a water-proof varnish for coating the wings of aeroplanes; it has been the cause of

several fatal industrial poisonings in aeroplane factories, being commonly known as "dope" poisoning.

The liver showed irregular fibrosis and cellular destruction, with very marked regeneration in one part, and almost complete absence of fat.

2. *Di-nitro-benzol Poisoning.*—The liver showed irregular fibrosis, very little degeneration of cells, much bile-duct proliferation and no fat. This was obviously an example of a very chronic form of toxic hepatitis.

3. *Tri-nitro-toluene Poisoning.*—The liver showed marked reduction in size, irregular fibrosis and very advanced fatty degeneration.

4. *Acute Yellow Atrophy.*—The liver was characterized particularly by absence of fat in the liver cells and marked fatty degeneration of the endothelial cells.

In August, 1916, Drs. Livingstone-Learmonth and Cunningham (2) described the symptoms and treatment of TNT poisoning among women workers in some of the large munition plants in England. This is the first comprehensive description that we know of. The symptoms are mainly due to the inhalation and ingestion of TNT dust. Exposure to the fumes alone might give the same effects, with this exception, however, that the irritating effects on the respiratory tract would be less.

The symptoms observed were divided into irritative effects due to action of the poison on the skin and mucous membrane, and toxic symptoms due to the degenerative hepatitis.

The former include nasal irritation with occasional epistaxis, lacrymation and smarting of the eyes, a sensation of constriction in the throat, and dry cough with yellow bitter sputum. Patients complained of a bitter taste in the mouth, of spasmodic griping pains in the abdomen, of acid eructations and often of vomiting. Early in the intoxication there was obstinate constipation followed by diarrhœa with tenesmus. The skin showed rashes of a cheiropompholix or eczematous type over the face and extremities, most prominent where sweating was greatest.

The toxic symptoms included nausea, vomiting, anorexia, jaundice, mental depression, blurred vision, and transient peripheral neuritis. The poisoning terminated in delirium or sometimes in coma or in convulsions.

REPORT OF CASE

The case observed was that of a white man, 24 years of age, who had always enjoyed good health. In April he started work in a munition plant devoted to the filling of shells with TNT.

He was employed in the mixing and stirring rooms, and his work consisted in dumping the flaked TNT into steam kettles stirring it as it melted, and filling shells with the molten substance. There was no TNT dust in the room.

When he had been at this work for seven weeks, he complained to the factory doctor of nausea and vomiting, slight abdominal pains and general weakness. Except for jaundice and tenderness in the epigastrium the physical examination revealed nothing.

He laid off work but grew gradually worse, and two weeks later was removed to a hospital, markedly jaundiced, vomiting persistently, prostrated and slightly delirious. There was no fever. He died in coma five days after admission.

The striking features of the case were the obstinate constipation, abdominal cramps, tenderness in the liver region, nausea and vomiting, prostration, and progressively deepening jaundice, the low muttering delirium, anuria, and terminal coma. Similar symptoms are common in delayed chloroform poisoning, phosphorus poisoning, acute yellow atrophy, Weil's disease, and certain forms of fatal malaria. In all these diseases the symptoms suggest hepatic failure due to extensive destruction of liver cells.

The autopsy was performed three days after death. As the body had been embalmed it would be of little value to give the entire autopsy protocol. The anatomical diagnosis was: Toxic hepatitis with extensive destruction of the liver parenchyma, toxic degenerative tubular nephritis; icterus; cloudy swelling of heart muscle; increased blood destruction in the spleen; and secondary anemia showing considerable polychromatophilia.

A striking feature was the intense jaundice. The skin of the entire body was a deep chrome yellow and all of the viscera were bile-tinged. The anatomical changes of chief interest were those in the liver, which I will describe in detail.

Gross Examination.—On opening the abdomen the free edge of the liver presents two fingers' breadth above the free costal margin. The organ is small and contracted. The serosa is smooth, glistening and free. On section the cut surface as a whole is grayish in color except for blotchy red areas where the embalming is imperfect. The region of the efferent veins is generally light red in color, soft in consistency and somewhat collapsed. The periphery of the liver lobule is greenish gray, hard and firm.

Microscopic Examination.—There is cloudy swelling of liver cells with extreme fatty degeneration followed by necrosis and autolysis of liver cells leading to extensive destruction of liver parenchyma. This destruction is so extensive that liver cells in their normal cord-like arrangement are no longer seen, except occasionally in areas around and near a portal space. In the middle and inner parts of the liver lobule around the efferent veins the liver cells are no longer recognizable. Around the periphery of the liver lobule there is extensive infiltration, chiefly of small lymphocytes with occasional wandering cells, but few polynuclears. The bile-ducts at the periphery of the liver lobules show extensive regeneration and proliferation. Throughout the liver tissue there are areas showing fine, greenish, granular pigment; in places this pigment is so abundant as to form cord-like masses, the so-called bile thrombi. There are numerous scattered areas showing hemorrhage, the tissue being filled with erythrocytes. Sections stained for fat show marked fatty degeneration of the liver cells, the few left being often filled with small fine irregular fat globules. The necrosis seen in the liver is so extensive and diffuse that it is quite impossible to ascribe to it any zonal distribution, but there is some evidence that it is more severe in the region of the efferent veins, the best preserved liver cells being usually in the region of the periportal space.

The picture is that of a toxic hepatitis, characterized by extensive cloudy swelling, fatty degeneration and necrosis of the liver cells, most marked in the region of the efferent veins but not distinctly zonal in character; lymphocytic perilobular infiltration; bile-duct hyperplasia; jaundice, and areas of extensive hemorrhage. The damage is beyond any known possibility of liver-cell regeneration.

References:

1. Spilsbury, B. H. Demonstration of a Series of Livers Affected by Toxic Hepatitis. *Lancet,* 1916, i, 999.
2. Livingstone-Learmonth, Agnes, and Cunningham, Barbara M. The Effects of Tri-nitro-toluene on Women Workers. *Lancet,* 1916, 261.

THE CLASSIFICATION OF DIPHTHEROID BACILLI BY FERMENTATION REACTIONS

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I wish to give merely a preliminary report on a series of sugar fermentations with about 60 different strains of diphtheroid bacilli. The work was begun in this laboratory by Mr. F. Eberson, who isolated most of the strains, and turned them over to me only when he left this country to take a position abroad. Considerable work has been done by others in the last few years on this class of organisms and I shall cite briefly some of the results obtained and theories advanced, especially in regard to the etiology of Hodgkin's disease.

In 1914 Bunting and Yates described the production of what appeared to be Hodgkin's disease in monkeys by the injection into the lymphatic glands of cultures of diphtheroid bacilli which were obtained from the glands of patients suffering from this disease. They were able to obtain the organism again from the glands of the monkeys inoculated and to transfer the disease from one monkey to another.

Billings and Rosenow in 1913 reported improvement in cases of Hodgkin's disease following the injection of diphtheroid vaccines.

Bloomfield, however, in 1915 obtained cultures of diphtheroids from lymph nodes not only in cases of Hodgkin's but in a large variety of other diseases involving these glands and in a few cases from normal nodes. Moreover, all agglutination and complement fixation tests with the serum of the Hodgkin's patients were negative and large doses of diphtheroid vaccine caused no reaction.

Fox confirmed these results and also showed that the fermentation reactions of different strains of diphtheroids varied greatly. Similar results were also obtained by Torrey. The strains with which I have worked have been obtained from almost all parts of the body, and have all apparently belonged to the indefinite diphtheroid group. For my sugar reactions I used saccharose, maltose, dextrose, dextrin, lactose and mannit in serum water with Andrade's reagent. My tests were repeated always twice and usually three or more times, and transfers were always made from tubes showing no acid formation to prove that growth had taken place. Frequent microscopical examinations of smears were made to rule out the possibility of contamination.

The sources from which my strains were obtained are as follows:

Lymph nodes in Hodgkin's disease.
Lymph nodes in pseudo-leukæmia,
Lymph nodes in chronic leukæmia,
Oral pus pocket,
Anal pus pocket,
Ascitic fluid,
Blood of indefinite febrile case,
Hypertrophied tonsils,
Bone marrow,
Urine,
Eye,
Nose,
Skin,
Brain in case of meningitis,
Urine in case of gonorrhœa,
Preiz Nocard bacillus from a horse,
Preiz Nocard bacillus from a sheep,
Preiz Nocard bacillus from a calf,
Acne pustule,
Ear, otitis media,
Appendix,
Blood in pneumonia,
Bile, cholecystitis.

Among the eleven strains obtained from cases of Hodgkin's disease are several, kindly sent to us by Bunting and Yates, and by Rosenow.

In the fermentation tests the strains divide themselves into eight different ferment groups. A large number form no acid, a few ferment all the sugars, and the remainder can be divided according to their sugar reactions into six different groups. In seven of these groups are one or more strains obtained from Hodgkin's glands. In the same way strains from the eye, the nose, etc., do not fall into one but into several groups. Several strains of the Preiz Nocard bacillus, however, all fall in the same group. It is now some time since these reactions were completed and I intend to repeat a number of them in order to see if they remain constant after protracted growth of the organisms on culture media and can be relied upon as a means of differentiation.

A number of Hodgkin's strains have been injected into rabbits, living and in large amounts and have absolutely no effect upon the animals. So far, I have been unable to stimulate the formation of agglutinins in rabbits by the injection of these organisms.

It seems very unlikely that any of the strains with which I have worked can have any etiological relationship to Hodgkin's although it is quite probable, as suggested by Bloomfield, that the organisms grow more readily in tissues already the seat of disease.

Discussion:

Dr. Krumwiede: There is one statement that I should like to comment upon, namely, that the negative fermentation tubes gave abundant growth on transfer. This method of checking negative results is frequently mentioned in recent bacteriological literature. It is, however, no indication that the bacterium cannot ferment the carbohydrate. I had, recently, a large series of strains from an outbreak of septic sore throat. In Hiss serum-water medium containing salicin, not one would ferment the salicin, though transfers gave abundant growth. If peptone were added, however, fermentation promptly occurred. Wolbach recently reported a series of streptococcus strains, many of which failed to ferment lactose in serum-water, although milk was coagulated. He checked the results by the presence of growth and drew certain conclusions from the results. Evidently the coagulation of the milk was due to acid production from the milk lactose, the milk being a medium more favorable for the development of the fermentative action of the cocci, and not to some other factor.

Dr. Lambert: Did you get the organism from all the cases studied? How many Hodgkin's glands did you investigate, and what percentage of cases with Hodgkin's disease showed this organism?

Dr. McBurney: A pretty large percentage. One gland had a large abscess, secondarily infected with staphylococci, but the diphtheroid was there and was very easily plated out. We had the same result with a gland from leukæmia.

Dr. Libman: What method did you use for the studies of acid production?

Dr. McBurney: We used the Hiss serum-water medium with Andrade's reagent.

Dr. Libman: Some years ago Drs. Celler, Sophian and myself were doing some work on streptococci, and we pointed out that the Hiss medium has not an active value for the study of acid production because bacteria do not grow readily in it. The results were better if peptone was added. We found that the best medium for the testing of acid production for bacteria is a fluid medium containing non-heated serum and carbohydrate; the next best was agar containing unheated serum and carbohydrate; the third best was a fluid medium containing carbohydrate alone, and the poorest medium was agar containing carbohydrate alone.

We came to the same conclusion as Dr. McBurney as regards the studies that had been reported in Hodgkin's disease. There is no doubt that organisms are found quite often in diseased lymph nodes and other non-healthy tissues. Apparently such organisms get used to living in such a tissue and then when cultures of them are injected into animals they have an affinity for similar tissue. In such instances we are dealing with the condition of latent microbism.

Dr. McBurney: In regard to the criticism of acid reactions, we tried to get differentiation between various strains; we did not try to get as many strains to form acid as possible. My object was merely to show that various strains are not identical.

Dr. Ewing: In any discussion of the bacteriology of Hodgkin's disease mention should be made of the work of Torrey, which has shown that several types of diphtheroid microörganisms are commonly present in the lymph nodes of this disease, none of which appears to have definite pathogenic action or deserve to be accepted as the exciting factor in the malady. He and Bloomfield have also shown that another microörganism, called *B. lymphophilus,* is constantly present in the nodes, while rarely found elsewhere, but again the indications are not satisfactory that it is the pathogenic agent in the disease.

Dr. MacCallum: I have not seen the results of Bunting and Yates. I would like to ask if they have seen anything in experimental animals which resembles the sharp, specific character of the involvement in Hodgkin's glands? If not, it seems to me that the analogy rests on an insufficient basis.

Dr. Libman: We found the organism in the tuberculous lymph nodes. There was one case in Boston in which it was found in acute leukæmia. Dr. Olitsky found the complement fixation negative in Hodgkin's disease. He never got complement fixation in human beings, though he did in animals.

Dr. McBurney: Bunting and Yates in their cases got enlargement of the lymph nodes by repeated injections in monkeys. A large number had secondary infections besides the diphtheroid bacillus. The sinuses after injection often did not heal, and their descriptions of the lesions do not correspond closely to Hodgkin's disease.

STUDIES ON THE PARATYPHOID GROUP AND ANTIGENIC AND CULTURAL DIFFERENCES IN STRAINS OF B. TYPHOSUS

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Early in my study of the paratyphoid and typhoid groups, cultural and antigenic variations among the strains within these groups were observed. Variations from the fixed types have been noted by others but no classification in the individual groups has to my knowledge been published. This preliminary report on the work attempts to show that such a classification may be made.

PARATYPHOID A

The cultural and antigenic investigation of twelve strains, isolated from human cases, has shown that paratyphoid A strains differ from one another in both respects. Tests were made for their ability to ferment dextrose, mannit, maltose, lactose, saccharose, xylose, rhamnose and inosite. Mr. J. S. Rice, of the Roosevelt Hospital laboratory, was my co-worker on inosite fermentation. The results of the fermentation, agglutination, and agglutinin-absorption tests are shown in Table I. "A" indicates acid formation in twenty-four hours; "G" indicates gas formation in twenty-four hours. Delayed reactions are indicated by small letters. The figures in parenthesis indicate the titer of the respective sera with the strain against which the animal was immunized. Two other paratyphoid B sera, not shown in the table, were also employed. They gave results parallel with those recorded.

Nine of the strains of paratyphoid A studied reacted identically in the fermentation and agglutination tests. Three other strains showed both cultural and antigenic differences from the typical strains and from each other. The use of xylose was suggested by Harding and Ostenberg[1] and recently extended by Krumwiede, Pratt and Kohn[2] who in a recent issue of the *Journal of Medical Research* have established the fact that the nonfermentation of xylose differentiates paratyphoid A and a part of the group which we speak of as heterogeneous paratyphoid from the other members of the paratyphoid-enteritidis group.

TABLE I

Paratyphoid A

	Agglutination				Fermentation							
	Typhi murium Serum (1–16,000)	Paratyphoid B Serum (1–16,000)	Paratyphoid A Serum (1–16,000)	Agglutinin Absorption	Dextrose	Mannit	Maltose	Rhamnose	Inosite	Xylose	Lactose	Saccharose
9 strains..............	0	1–400 or below	1–8,000 to 1–1,600	+	AG	AG	AG	Ag	0	0*	0	0
Strain No. 167.........	0	1–2,000	1–8,000	+	Ag	Ag	Ag	Ag	0	0	0	0
Strain No. 322.........	0	1–2,000	1–8,000	+	AG	AG	Ag	Ag	0	0	0	0
Strain No. 168.........	0	1–400 or below	1–2,000	0	AG	AG	AG	Ag	0	0	0	0

PARATYPHOID B

The twenty-five paratyphoid B strains studied were isolated from human cases. The majority differed culturally from the A strains in that they produced gas on xylose and on inosite. All of these strains with one exception (No. 169) agglutinated well in paratyphoid B anti-serum. So far as tested they agglutinated also in a typhi murium serum. All failed, however, to absorb the agglutinins from the latter. The absorption tests carried out with two paratyphoid B sera brought out interesting differences between the strains, which are shown in Table II. One group absorbed the agglutinins from both sera. A second absorbed them from the "army" serum but not from the "Seeman"; and conversely, one strain was negative with the "army" serum and positive with the "Seeman." A fourth group failed to absorb the agglutinins from either. On the basis of the plus or minus reactions—that is tests which showed a very feeble agglutination with the homologous strain after absorption—three other intermediate antigenic types may be distinguished.

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* It is interesting to note that eight strains of paratyphoid A developed a very small amount of gas in xylose broth, though no acid was formed. On account of the absence of acid we have tabulated the fermentation as negative.

None of these strains agglutinated in an antiserum for paratyphoid A above the zone of group reactions.

TABLE II

Paratyphoid B

	Agglutinin Absorption			Fermentation								
	Paratyphoid B, Army	Paratyphoid B, Seeman	Typhi murium	Dextrose	Mannit	Maltose	Rhamnose	Inosite	Xylose	Lactose	Saccharose
12 strains	+	+	o	AG	AG	AG[1]	AG	G	AG	o	o
1 strain	+	+	o	AG	AG	AG	AG	o	AG	o	o
2 strains	+	o	o	AG	AG	AG	AG	G	AG	o	o
1 strain	+	o	o	AG	AG	AG	AG	G	A	o	o
2 strains	+	±	o	AG	AG[2]	AG	AG	G	AG	o	o
1 strain (No. 169)	o	+	o	AG	AG	AG	AG	G	AG	o	o
4 strains	o	o	o	AG	AG[3]	Ag	AG[4]	o	AG[5]	o	o
1 strain	±	o	o	AG	AG	AG	AG	o	AG	o	o
1 strain	±	±	o	AG	AG	AG	AG	G	AG	o	o

[1] One strain fermented maltose only after forty-eight hours.
[2] One strain fermented mannit only after forty-eight hours.
[3] One strain gave delayed fermentation on mannit.
[4] One strain gave acid only.
[5] One strain gave fermented xylose only after forty-eight hours.

B. TYPHI MURIUM

Five strains of bacilli from mouse typhoid were studied with the results shown in Table III. There are at least two groups represented, one which gives positive agglutination and absorption reactions with the serum used, and another which does not. The former may be further subdivided on the basis of the inosite fermentations. The agglutinating group ferments xylose slowly (4–5 days).

TABLE III

B. typhi murium

	Agglutination				Fermentation				
	Paratyphoid A Sera (1–16,000)	Paratyphoid B Sera (3 sera) (1–16,000)	Typhi murium[1] Serum (1–32,000)	Absorption Typhi murium Serum	Dextrose, Mannit and Maltose	Rhamnose	Xylose	Inosite	Lactose and Saccharose
Strain W.......	1–200	4,000–8,000	1–32,000	+	AG	AG	ag	0	0
Strain P & S....	1–200	4,000–8,000	1–32,000	+	AG	AG	ag	G	0
Strain 138 K....	0	+	1–32,000	+	AG	AG	ag	G	0
Strain 13.......	1–40	0	0	0	AG	AG	AG	0	0
Strain K.......	1–100	0	0	0	AG	AG	AG	0	0

B. CHOLERÆ SUIS

Seven strains of *B. choleræ suis* were studied. Variations noted in the fermentation reactions are shown in Table IV. One strain (112 K) produces acid but never gas. All but one strain agglutinated in paratyphoid B serum and also in an antiserum for *B. typhi murium* not shown in the table. They failed, however, to absorb completely the agglutinins from these sera and in this respect differ sharply from the typical members of these other groups. The one strain which failed to agglutinate is evidently a type distinct from the others. The remaining four all showed slight individual differences in the fermentation tests.

TABLE IV

B. choleræ suis

	Agglutination	Fermentation					
	Paratyphoid B Serum (1–16,000)	Dextrose	Mannit	Maltose	Rhamnose	Xylose	Lactose, Saccharose and Insonite
Strain No. 332 K.L.......	0	Ag	AG	Ag	Ag	Ag	0
3 strains...............	1–8,000	Ag	AG	Ag	Ag	Ag	0
Strain No. 112 K........	1–8,000	A	A	A	A	a	0
Strain No. 334 K.........	1–8,000	Ag	Ag	Ag	a	0	0
Strain No. 120 K........	1–8 000	Ag	Ag	Ag	A	Ag	0

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[1] Twenty-five paratyphoid B strains, six strains of *B. choleraæ suis* and two *B. abortus* which were tested, all failed to absorb the agglutinins from this serum.

B. ABORTUS

The two strains of *B. abortus* which we used gave typical cultural and agglutinative reactions. The failure to absorb out typhi murium and paratyphoid B serum showed, however, that they differ antigenically from six of our seven groups of paratyphoid B and from *B. typhi murium*.

HETEROGENEOUS PARATYPHOIDS

The twenty-one strains of paratyphoid included in this group came from a variety of sources, but in no case has proof been found that they are the cause of paratyphoid fever. Several of the cultures were stock laboratory strains which have no history. Isolations from stools were made in cases of nephritis, German measles, paratyphoid B and pyelitis. Several strains were obtained from urine. Two isolations, one from the blood and the other from the urine, were made from a surgical case. Recently I have isolated eight more such strains, one from a paratyphoid A case, one from a dysentery case from which the bacillus Rosen was also isolated, and six from the stool of normal individuals.

Uhlenhuth and Hübener obtained an organism from a case of meat poisoning which was similar to paratyphoid, but did not agglutinate in sera of the fixed types. This organism, which they called paratyphoid C, was agglutinated by the patient's serum and also by an enteritidis serum. Messerschmidt, Babes and Feodorasco, Arzt and Boese, and Sieffert and others have also found these paratyphoid-like types.

Our organisms reacted culturally like typical paratyphoid bacilli, all producing acid and gas very actively on dextrose, mannit and maltose, and being negative in lactose and saccharose They differ from the fixed types in their non-agglutination in paratyphoid A or B sera. Three sera made from strains belonging to this group reacted with the homologous strain only and showed no cross agglutination.

SUMMARY

The fermentation reactions characteristic of the paratyphoid group are the production of gas and acid from dextrose, mannit, maltose, and rhamnose, and the failure to attack lactose and saccharose. A number of exceptions were noted in this series. One strain produced only acid on dextrose, mannit, and on maltose. On rhamnose three cultures reacted in this atypical manner, producing acid but no gas. Considerable variation was also noted in the speed of gas production and slow fermentation was characteristic of the hog-cholera strains.

On xylose, paratyphoid A is negative, while paratyphoid B, *B. typhi murium*, and *B. choleræ suis* ferment with acid and, with few exceptions, with gas production also. This gives a convenient fermentative differentiation.

On inosite, paratyphoid A, *B. choleræ suis*, and *B. abortus* are negative; while paratyphoid B and *B. typhi murium* each contain a fermenting and a non-fermenting group.

The results of the agglutination reactions are more difficult to summarize. It is evident, however, that ten of our twelve strains of paratyphoid A reacted alike and that thirteen out of twenty-five strains of paratyphoid B reacted alike, as did three out of five strains of *B. typhi murium* and six out of seven strains of *B. choleræ suis*.

In each of these groups, however, are certain strains which show differences in their behavior toward immune sera which set them apart. The reactions suggest that there may be as many as six other antigenic types among our paratyphoid B cultures. A number of the remaining strains we hope to classify more definitely when we obtain homologous agglutinating sera.

B. TYPHOSUS

In my investigation of the *B. typhosus* I studied the cultural and antigenic properties of thirty-one strains coming from different sources.

Cultural Reactions.—I can at this time report the reactions upon eight fermentable substances: dextrose, mannit, maltose, xylose, lactose, saccharose, rhamnose and inosite. In every case where an atypical reaction occurred the test was repeated and not accepted unless checked, and the possibility of contamination eliminated by plating out the culture.

The most marked cultural variation occurred in the xylose fermentation. Of the thirty-one strains, twenty-two produced acid in twenty-four hours, three acted slowly fermenting sufficiently for a positive reading on the third day, and six remained negative after prolonged incubation. One of these is the Rawlings strain. Four of them are atypical antigenically as will appear later.

The strains were uniformly negative on rhamnose and inosite. That the non-fermentation of rhamnose differentiates *B. typhosus* from all the classified members of the paratyphoid-enteritidis group was pointed out by Uhlenhuth. Twenty strains reacted typically producing prompt acid fermentation in dextrose, mannit and maltose and failing to ferment the other sugars. Five other strains showed delayed fermentation in one or another of the sugars as indicated in Table V.

TABLE V

Cultural Variations in Strains of B. typhosus

No. of Strains	Dextrose	Mannit	Maltose	Lactose	Saccharose	Xylose	Rhamnose	Inosite	Litmus Milk	Antigenic Group
20	A	A	A	0	0	A	0	0		I*a*, I*b*, III*d*
1	A	a	A	0	0	A	0	0	alk. 5 d. acid	II
1	A	a	A	0	0	A	0	0		III*b*
1	a	A	a	0	0	a	0	0		I*b*
6	A	A	A	0	0	0	0	0		I*b*, II*c*, III*a*
2	A	A	A	0	0	a	0	0		I*b*

Agglutination Reactions.—The agglutinations were carried out with nineteen typhoid strains on four antityphoid sera before the more recently acquired organisms had been isolated or received. Two strains failed to agglutinate in one of the sera used and a third did not agglutinate in any. The remaining organisms could be further divided into two classes on the basis of the strength of their reaction.

Absorption Reactions.—It is difficult to make a classification on the basis of the intensity of the agglutination reactions though the differences brought out were extremely suggestive. For a further study of these differences, absorption tests were resorted to which were carried out in the following way. The sera were diluted to one eighth of the highest dilution in which agglutination occurred with the homologous strain. Thus, in a serum that had a titer of 1/16,000 an initial dilution of 1/2,000 was made. One half c.c. of this was absorbed with an entire slant agar culture of *B. typhosus* suspended in 0.5 c.c. of isotonic salt solution. This was incubated at 37.5 degrees for two to four hours and kept at ice-box temperature for four days. Whenever the supernatant fluid became clear, dry antigen was added and the tube again incubated. The organisms were then centrifuged off, a half c.c. of the supernatant fluid now in a dilution of 1/4,000 was tested for agglutinins with the homologous strain. This gave a final dilution of 1/8,000. Wherever there was partial or incomplete absorption, a sharp agglutination occurred.

Thirty strains were tested for their ability to absorb agglutinins from each of these four sera. Nineteen of them absorbed all agglutinins from each of the sera and four other strains were positive on three sera and negative in one. The other seven fell into five different classes as shown in Table VI.

The relationship between the antigenic and cultural characteristics of these strains is indicated in the right-hand column of Table V. Regarding this relationship it would appear that

TABLE VI

Antigenic Variations in Strains of B. typhosus

Division	Sub-grouping	No. of Strains	Agglutination in Typhoid Serum	Absorption Tests			
				Serum R. I.	Serum Rawlings	Serum P. and S.	Serum Novy
I........	*a*	4	+	+	+	+	−
	b	19	+	+	+	+	+
II.......	..	1	−	−	−	−	−
III......	*a*	2	±	−	−	−	−
	b	1	+	−	−	−	+
	c	2	+	±	+	−	−
	d	1	+	+	±	−	+

they are to a large extent independent of one another. Yet there is a tendency for the antigenically atypical organisms to be culturally atypical, and the fact stands out that with one exception, the organisms that were culturally typical belong to one of the two typical antigenic groups.

SUMMARY

Culturally, six of the typhoid strains studied differed from the typical races in failing to ferment xylose. Five other strains fell into four separate classes on the basis of the speed with which they fermented the test substances.

Antigenically, the majority of the strains reacted alike in absorbing the agglutinins from all the sera used. However, in this comparatively small series, eleven strains were found which fell into six different groups when classified by absorption tests.

It is not claimed that these studies have separated typhoid bacilli definitely in six cultural and in seven antigenic groups. They show, however, that cultural variations do occur and that a classification is possible on the basis of the absorption tests. Such a grouping will probably be of importance in typhoid prophylaxis, as a vaccine prepared from a single strain may not give complete protection.

The work will be continued with a larger series of strains in an effort to arrive at a more definite classification.

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A CASE OF POLYPOSIS OF THE SMALL INTESTINE ASSOCIATED WITH CARCINOMA

HENRY R. MULLER, M.D.

(From the Department of Pathology, Cornell University Medical College)

The material which forms the basis of this report is the terminal three feet of the ileum, with the exception of two or three inches next the cæcum, which was removed at operation by Dr. Steedly of Spartanburg, S. C. The patient was a man seventy-four years old. He had suffered from diarrhea and indigestion for 25 or 30 years, and lately, in addition, from chronic obstruction. On palpating the abdominal wall, the affected coils of intestine gave the impression of a solid tumor in the lower umbilical and right inguinal regions. At operation the mesentery was found to be very much thickened, and the affected coils were largely adherent. It was thought at operation that all the affected coils had not been removed.

On gross appearance the intestine presents a thickening of the walls, gradually increasing from above downward. The whole intestinal wall is very firm and rigid. The mucosa of the first eight inches of the specimen appears relatively normal, but rather suddenly the valvulæ conniventes become obliterated and small hard pin-point nodules appear scattered over the surface. Passing down the intestine, very numerous polypoid masses are seen. They average about 4 or 5 mm. in diameter. Some are flat and sessile, others pedunculated. Where the process is farthest advanced there are four or five somewhat longer masses, about 1 cm. across; and 8 cm. from the cæcal end there is a lobulated mass as large as the end of the little finger. The mucosa between the polyps is thick and hard. Nearer the cæcal end the mucosa is so very much thickened that the lumen of the intestine is practically obliterated and is represented merely by a narrow slit. On cross-section it is seen that the muscle coats are greatly thickened, and that scattered throughout the wall and beneath the peritoneum are hard grayish nodules varying in size.

On microscopical examination the sections show that the entire specimen is the seat of a marked chronic enteritis. The polypoid masses, taken from the part where the lesion is least advanced have the structure of adenomata, with the epithelial cells, however, showing evidences of beginning malignancy by the fact that the nuclei are hyperchromatic, and the cells show many vacuoles. These polyps also are the seat of chronic inflammation. Where the lesion is most advanced the sections show a fully developed diffuse adenocarcinoma with nests of carcinoma cells in the lymphatic spaces of the submucosa, and in many places the carcinoma has extended through the muscle coats.

To summarize then, this is a case of diffuse polyposis affecting the ileum, associated with chronic enteritis and carcinoma. The fact that it occurs in an individual seventy-four years old is interesting, since the majority of cases reported have been in much younger individuals, from twenty to forty years.

The answer to the ever-recurring question of the relationship of the three processes—enteritis, adenoma and carcinoma—to one another is difficult to determine definitely in this case. The sharp line of advance of the polyps showing beginning malignancy would indicate that the stimulus for the formation of both polyps and carcinoma were the same. Then, as Lubarsch thought possible in his cases, the chronic enteritis could be regarded as being, at least in part, their underlying cause.

SITUS INVERSUS

H. S. MARTLAND, M.D.

(*From the Pathological Laboratory of the City Hospital, Newark, N. J.*)

The specimen consists of the thoracic and abdominal viscera removed from a female, thirty-two years of age, who died as the result of carbon-monoxide poisoning, the gas having been taken with suicidal intent. The specimen was obtained through the courtesy of Dr. Warren, coroner's physician of Newark.

The transposition is complete and absolute. It involves single organs which belong to a certain side or location as heart, liver with gall-bladder, stomach, pancreas, spleen, colon and appendix and paired organs as the lungs and kidneys. The whole viscera form a complete mirror-like inversion of the normal condition.

The organs are otherwise, anatomically speaking, normal in appearance and there is no other associated congenital anomaly or fault.

The large vessels going to and from the base of the heart are completely transposed. The arch of the aorta, for instance, turns to the right and the thoracic aorta descends over the right side of the vertebral bodies. The three large arteries arising from the arch are completely transposed. There is no other circulatory anomaly present, which is important when we consider that one of the two romantic theories regarding the etiology of this condition is based upon anomalies of the circulation of the early main current of blood.

The brain is quite normal in appearence from the anatomical standpoint. We are unable to ascertain whether the woman was a right- or left-handed individual nor do we know whether she was one of a twin pregnancy.

EXPERIMENTAL STUDIES OF CHOLESTEROL METABOLISM

J. HOWARD MUELLER, PH.D.

(From the Department of Pathology, College of Physicians and Surgeons)

It is at present generally accepted that cholesterol, which is apparently a primary cell constituent of animals, is not synthesized from simpler substances by the animal organism, but is taken in the food, and has its ultimate origin in the phytosterols, a group of closely related substances occurring in plants. It must be said, however, that conclusive evidence against the animal synthesis has never been brought—that there is at least a chance that as our knowledge of the synthetic capabilities of animal cells gradually widens, it may be found that under some circumstances a synthesis of cholesterol may also take place. On the other hand, it has been shown definitely that it is absorbed from the intestine, since feeding on a cholesterol-rich diet leads to an increased amount in the blood and organs. From this point on, nothing is known of its intermediate metabolism. Lifschutz has brought considerable evidence to show that it is partially oxidized in the body and that the cholic acid radicle of the bile salts is produced from it. This has not as yet been confirmed and others hold that it is not destroyed in the body, but is excreted in the bile, perhaps (as Rothschild believes), as a result of a specific activity of the Kupffer cells. Reabsorption from the intestine follows; the degree depending, perhaps, upon the needs of the body and the amount supplied in the food.

It is evident that we have here only the barest outline of the process, quite lacking in all details. In order to be able to understand the disturbances in the normal metabolism of cholesterol which seem to occur in cholelithiasis, fevers, pernicious anemia, and other conditions, it is essential to have more detailed information on the subject and with that end in view, the work which is to be described was undertaken.

The question of the mechanism of the absorption is logically the first to be considered. From the similarity of cholesterol and its fatty acid esters with the neutral fats, absorption might be expected to take place from the intestine by way of the lymphatics. This was readily found to be the case in dogs. Chyle from a thoracic duct fistula after feeding eggs, or pure cholesterol dissolved in cotton-seed oil, showed a much higher cholesterol content than a specimen from the same dog before feeding. Tables I and II present two typical cases.

TABLE I

Fistula Completed at 1 p.m.

Chyle		Free Cholesterol		Cholesterol Esters		F : T[2]
Time Collected	Amount	Amount		Amount		
p.m.	*gm.*	*gm.*	*per cent.*	*gm.*	*per cent.*	
1.00	13.4	0.0049	0.036	0.0088	0.066	35.29
5.30[1]	15.2	0.0060	0.039	0.0100	0.066	37.62
7.30	9.6	0.0059	0.062	0.0115	0.119	33.91
8.30	10.0	0.0088	0.088	0.0207	0.207	29.83
9.30	9.8	0.0111	0.113	0.0270	0.276	28.32

[1] Time of feeding.

[2] Figures in this column indicate the percentage of the total cholesterol which is free.

TABLE II

Fistula Completed at 1.30 p.m.

Chyle		Free Cholesterol		Cholesterol Esters.		F:T
Time Collected	Amount	Amount		Amount		
p.m.	*gm.*	*gm.*	*per cent.*	*gm.*	*per cent.*	
4.30[1]	10.7	0.0026	0.024	0.0045	0.042	36.36
6.45	9.8	0.0093	0.095	0.0133	0.136	41.13
8.45	9.7	0.0065	0.067	0.0100	0.103	39.41
a.m.						
12.30	10.0	0.0067	0.067	0.0106	0.106	38.73

[1] Time of feeding.

It will be noted that both the free and combined cholesterol increase and that the proportion between the two remains nearly constant. This is true when free cholesterol is fed as well as when cholesterol oleate is given, and apparently represents an adjustment of the proportion of the free and combined to that normal to the chyle and also to the blood plasma.

Since nothing has been known of the interchange between the free and combined forms of cholesterol in the body, this change during absorption seems to offer a particularly fortunate opportunity for more detailed study. There are conceivably three agencies to be considered, namely the lumen of the gastro-intestinal tract, its mucosa, and the mesenteric lymph nodes, and each of these has in turn been investigated.

TABLE III

		Free	Combined	F:T
		per cent.	*per cent.*	
Dec. 30.	Biliary fistula made.			
Jan. 1				
11.30 a.m.	Thoracic duct fistula completed. Chyle taken..............................	0.021	0.028	43
1.30 p.m.	Chyle taken. Fed small piece of meat.			
1.45 "	Fed four raw eggs.			
5.00 "	Chyle taken, very milky..............	0.056	0.038	60
6.00 "	" " " "	0.058	0.037	61

By excluding experimentally certain digestive juices from acting upon cholesterol fed to dogs, and studying the changes in the chyle during absorption, certain information has been gained. Normal absorption was obtained by injecting a solution of cholesterol in cotton-seed oil directly into the duodenum, thus ruling out gastric digestion. The latter is therefore probably not essential for its absorption. After ligating and severing the common bile-duct and making a gall bladder fistula, a greatly modified absorption was obtained in several dogs. The following table will illustrate this change.

The total increase is seen to be much less than in normal absorption. In addition, over 60 per cent. of the total cholesterol is free, as opposed to 30 to 40 per cent. ordinarily found. Evidently the bile plays rather an important part in cholesterol absorption normally.

In a number of dogs, too, the pancreatic ducts were ligated and cut, leaving the organ itself in situ, with blood supply as nearly intact as possible. Absorption of cholesterol through the chyle in such dogs was practically nil, pointing to the importance of the pancreatic enzymes in the process.

TABLE IV

	Titration	Cholesterol		F : T
		Free	Combined	
	cc.	*gm.*	*gm.*	
Free cholesterol 0.02 gm., fresh	3.8	0.0212	0.0006	97
" " 0.02 " incubated	14.8	0.0104	0.0102	50
Cholesterol oleate 0.04 gm., fresh	3.5	0.0062	0.0058	52
" " 0.04 " incubated	17.0	0.0032	0.0100	24

Certain experiments were undertaken to see whether there could be demonstrated an action in vitro by the enzymes of the pancreas and intestinal mucosa, alone and in the presence of bile, upon cholesterol in the presence of fatty acids, and upon cholesterol oleate. Although in all such experiments neutral fat present was readily split as evidenced by a titration of the fatty acids formed, no splitting of cholesterol oleate was ever observed. On the other hand, when a suspension of pancreas in dilute glycerol was used, free cholesterol was found after incubation to have become to a marked degree converted into cholesterol esters. In parallel experiments in which intestinal mucosa and a suspension of lymph nodes were used, in both of which an active lipase was present, no such change took place, and it seems that this may explain, to some degree at least, the necessity for pancreatic juice in the absorption of cholesterol. This esterification is illustrated in Table IV.

In another series of experiments the intestinal mucosa of dogs was carefully washed, removed and analyzed. Several analyses were made on dogs which had received no food for twenty-four hours, and a number on dogs which had been fed cholesterol two or three hours before being killed.

TABLE V

	Duodenum			Jejunum			Colon		
	Free	Ester	C : T[2]	Free	Ester	C : T[2]	Free	Ester	C : T[2]
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*	
24 hrs. fasting........	0.0109[1]	None	—						
24 " "	0.0092[1]	0.0006	6.1						
24 " "	0.0131	None	—	0.0098	None	—	0.0116	0.0006	4.9
Fed three eggs........	0.0101	0.0021	17						
Fed 300 cc. of milk with 2 gm. cholesterol in 8 cc. of cottonseed oil..	0.0111	0.0017	13						
Fed three eggs........	0.0194	0.0061	24	0.0215	0.0064	23	0.0131	0.0014	9.7
Fed two eggs.........	0.0079	0.0014	15	0.0105	0.0012	10	0.0071	0.0002	2.7

It is evident that there is little combined cholesterol normally present in the intestinal mucosa of dogs, while a considerable amount is found during absorption, when the cells and lymphatics are filled with lipoidal material.

From these observations it seems clear that the esterification of the free cholesterol must take place in the small intestine under the influence of the pancreatic juice, perhaps aided by the bile. The esters are then absorbed as such. No evidence that the mesenteric lymph nodes exert an influence on cholesterol during its absorption could be obtained although the possibility of such an influence was not satisfactorily excluded, nor was it determined how the increase in free cholesterol always seen in the chyle after feeding takes place, and is regulated.

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[1] A mixture of duodenum and jejunum mucosa analyzed.

[2] Figures in these columns indicate the percentage of the total cholesterol which is combined in the form of esters.

From the time that the cholesterol is poured by the chyle into the blood stream, its changes in form and activities are still largely unknown. Only about one fourth to one third of the total cholesterol of the plasma is free, the rest being in the form of fatty acid esters. In the red blood cells, however, there is normally no combined cholesterol, and in practically all the organs the cholesterol is largely free, as is that excreted by the bile. Where the esters of the chyle and the blood plasma are again split has not been determined. Schultz and Cytronberg claim to have found an enzyme (cholesterase) in the red blood cells and in mixtures of the latter with liver pulp which actively split them. These observations have remained without confirmation and in a number of experiments I was able in no case to obtain results at all comparable with theirs. This splitting of cholesterol esters which undoubtedly occurs in the body is perhaps a function of all cells so intimately bound up in the delicately balanced life processes as to be insusceptible of demonstration under the conditions of autolysis. Perhaps experiments can be devised which may be more successful. I hope to be able to report later on this intermediate metabolism of cholesterol.

In regard to the excretion, little can be said beyond the fact that it must be controlled by a very delicately balanced mechanism. Reabsorption of biliary cholesterol doubtless takes place along with cholesterol of the food. There is, however, a certain amount eliminated by the feces at least some of which probably comes from desquamated cells of the mucosa of the colon. In a dog fed on a fixed diet the feces for a five-day period contained about 0.6 gram cholesterol. In a second five-day period, in which 0.3 gram cholesterol per day was added to the food, 0.8 gram was excreted. In a third five-day period, without the addition of any cholesterol to the food, 0.9 gram was excreted, and in a fourth period, in which one gram of cholesterol oleate was added to the food each day, 1.3 grams were excreted. Evidently a very considerable amount in excess of that ordinarily present in food, can be absorbed, but the complete absorption of all the food—and biliary—cholesterol which would doubtless lead to various forms of pathological infiltrations with arteriosclerosis or cholelithiasis is in some way prevented normally.

Up to the present stage, perhaps, these experimental studies have added little or nothing to our understanding of pathological conditions. Possibly further work along the lines indicated may be more fruitful. There is, however, one statement in the literature to which reference is frequently made, upon which some light may be thrown. This is the belief expressed by Faust and Tallquist that the severe anemia of the pernicious type often occurring in individuals harboring the *Bothriocephalus latus* may be due to cholesterol oleate occurring in the body of the worm. The idea is at first view somewhat surprising since free cholesterol is well known to be an anti-hemolytic substance, and that a simple ester, which indeed occurs in very considerable quantities normally in the plasma, should be so strongly hemolytic and toxic as to cause a severe anemia when present in the rather small amount found in the worm is at least peculiar. When examined more closely their conclusion is found to be based upon the observations that the ether extract of the dried worm was hemolytic; that after saponification, oleic acid was obtained and cholesterol was identified; and further, that no glycerol was present and hence that the original substance must have been cholesterol oleate. When cholesterol oleate was fed to a dog, sodium oleate, a hemolytic soap, was identified in the chyle, but no cholesterol was present. It is argued from these findings that cholesterol oleate is potentially toxic, since it is split in the intestine, the cholesterol excreted and the oleic acid combined with sodium to form a soap which, when absorbed, acts as a hemolytic substance. The objections to such a view are obvious in the light of our present understanding of cholesterol absorption. Manifestly their failure to recognize cholesterol in the chyle was due to an error in technique, due perhaps to the imperfect analytical methods at that time available. Faust later undertook the study of the effect of feeding large quantities, 10 to 20 grams daily, of oleic acid to dogs. In one case a slight decrease in red count, from 7,000,000 to 5,000,000, increasing again to 6,000,000 resulted in the course of six months, and in the other case no decrease was recognized. The hemoglobin, however, fell considerably in both dogs, while in *Bothriocephalus latus* anemia it is relatively high. This would seem to show pretty conclusively that the oleic acid which might be set free from a small amount of cholesterol oleate is entirely inadequate to account for the severe anemia seen in some cases of bothriocephalus infection. It may be added that in an experiment recently carried out, a dog was fed daily a gram of cholesterol oleate for a week, and then for an equal time received daily intravenous injections of a gram of cholesterol oleate dissolved in a little sesame oil. The red blood count of this animal remained practically constant throughout at 6,500,000, thus pretty well ruling out any severe hemolytic effect by the compound.

Discussion:

DR. MACCALLUM: Dr. Müller's work on this most important subject seems to throw the first real light upon the nature of the absorption of cholesterol. Since this substance has come to be recognized as playing such an important part in many diseases as well as in many normal processes, I hope Dr. Müller will continue the work and perhaps later be able to tell more of the modifications which the esters undergo in the tissues.

DR. WOOD: I am glad that the Faust-Tallquist idea has at last been settled. It never seemed to me likely that a mere trace of oleate could have so extensive a hemolytic effect, but much more probable that the cause is a far more toxic substance. It is interesting to see how much work has been done on cholesterol even though the structural formula has by no means been made out. At present much energy has been focussed on the subject and with the more accurate analytic methods which are available at the present, much more will no doubt soon be accomplished.

CARCINOIDS OF THE SMALL INTESTINE AND THEIR RELATIONSHIP TO THE SO-CALLED CARCINOMA OF THE APPENDIX

B. M. VANCE, M.D.

(From the Pathological Department, Bellevue Hospital, Charles Norris, M.D., Director)

The six cases to be presented, consist of four minute primary epithelial tumors of the small intestine and two similar growths of the appendix. These neoplasms are remarkable in that they show a histological picture similar to that of a very malignant carcinoma, but their mode of growth is rather more benign than the microscope would indicate. The name "carcinoid" has been applied to them because of this very peculiarity.

The four specimens from the small intestine were discovered, quite by accident, in autopsies at Bellevue Hospital. The patients were elderly individuals, with ages varying from forty-nine to sixty years. In every case the neoplasm had no influence on the patient's health, and death was due to some other condition.

The tumors were solitary, and in size varied from a wheat grain to a hazelnut. They took their origin from some structure in the submucous layer and were invariably situated on the side of the gut opposite the mesenteric attachment.

No metastatic nodules could be found in other organs.

Case I was a man sixty years of age. The cause of death was coronary sclerosis. A firm, gray white nodule, the size of a wheat grain, was found in the ileum one foot from the ileo-cæcal valve.

Case II was a man of forty-nine years who died from an empyema of the right pleural cavity. There was a firm, white tumor, as large as a bean, in the middle of the small intestine.

Case III was a woman of fifty-eight years. In addition to the carcinoma of the thyroid which caused her death there was a firm, white nodule, the size of a hazelnut, situated in the ileum about a yard from the ileo-cæcal valve.

Case IV, a man forty-nine years of age, died of pulmonary tuberculosis. A small, white nodule, the size of a bean, was found in the small intestine a short distance from the cæcum.

The histological pictures presented by these four different tumors were similar in all essential features, but disclosed a few minor variations. The growth in Case I was composed of short columnar cells, containing large, ovoid, centrally placed nuclei, clumped together as solid plugs in alveoli of different sizes. In some of the spaces, the cells near the rim, were arranged in a regular palisade, and there was some attempt at rudimentary gland formation (Fig. 1). The stroma was composed of strands of non-cellular fibrous tissue, containing a very moderate vascular supply. Unfortunately the specimen was not stained by Van Gieson's stain to determine the presence of non-striated muscle in the stroma.

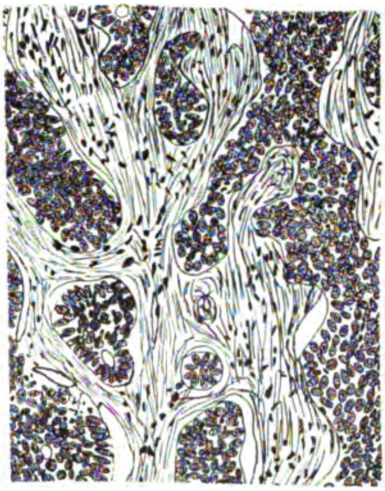

Fig. 1. Case I.

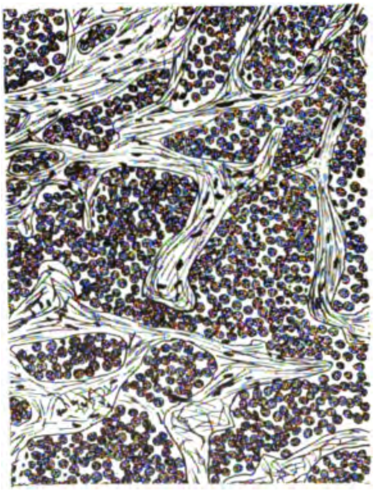

Fig. 2. Case II.

Cases II, III and IV showed the same histological characteristics, and one description will serve for the three. The tumor cells were globular or polyhedral, containing large, spherical, centrally placed nuclei. They were arranged in solid plugs in alveoli of different sizes and showed no attempt at gland formation (Fig. 2). The stroma was composed of dense, non-cellular fibrous tissue and quite similar to Case I, in this respect. Case II was stained to demonstrate smooth muscle fibers in the stroma, but none could be found.

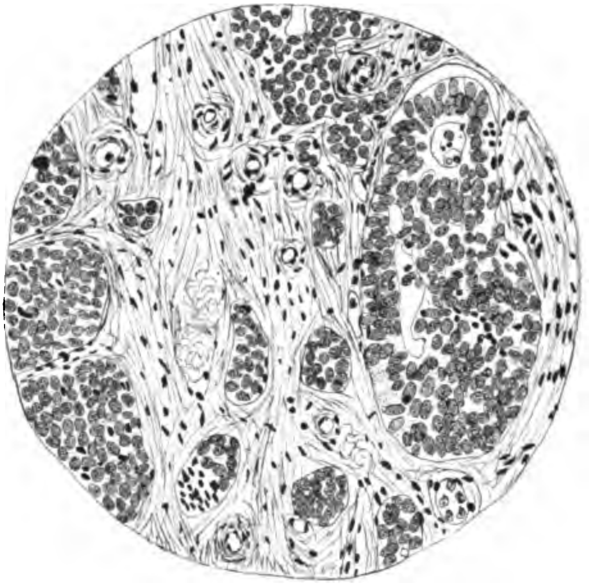

Fig. 3. Case V.

In every instance, the primary site of the tumor was the submucous layer. The growth in Case I showed no infiltration into the surrounding tissue around the nodule. In Cases II, III and IV, the neoplasm had penetrated the muscularis mucosæ, and was destroying the mucous membrane. In Case IV, there was a slow infiltration of the tumor tissue, backward through the muscular layer and sideways into the lymphatics of the submucosa.

The two epithelial tumors of the appendix were very similar to the carcinoids of the ileum, but unlike them were taken from young individuals. Both patients were operated upon for appendicitis and the presence of the neoplasm was not suspected before operation.

Case V. The patient was a woman twenty-three years of age. The appendix was 7 cm. in length, and showed a normal proximal portion, 4.5 cm. long and 6 mm. in diameter. The distal end was a solid, foot-ball shaped, mass of gray white tissue, about 2.5 cm. by 9 mm. by 9 mm. Under the microscope, the distal end was composed of tumor tissue supported by a thick network of fairly vascular connective tissue. The alveoli were large and contained short columnar cells, possessing large, centrally placed ovoid nuclei. The outside rim of cells were in palisade arrangement along the alveolar wall, while the central mass of cells were collected together in solid clumps (Fig. 3). The neoplasm had destroyed the submucous layer and the mucous membrane, and was penetrating the muscular zones. No secondary metastatic tumors were discovered at operation.

Case VI.[1] The appendix removed from a man, eighteen years old, was somewhat larger than that in Case V, but disclosed the same features on gross examination. Microscopically, the tumor consisted of large polyhedral cells, containing large spherical nuclei. They were supported by a thick, connective tissue stroma, and clumped together in solid plugs. The neoplasm had not only destroyed the mucous and submucous layers, but had penetrated to the subperitoneal coat. No metastases, however, could be found in the neighboring lymph nodes or elsewhere.

In both these tumors of the appendix, it is probable that the submucous layer was the point of origin, though this would be difficult to demonstrate in neoplasms of this size. It is evident, however, that in both the cases of the small intestine and the appendix, we are dealing with the same neoplastic type. Most observers now recognize this fact, and identify the so-called carcinoma of the appendix in young people with the carcinoid of the small intestine found in older individuals.

The difference in the ages of the two sets of cases is not easy to explain, but the obvious submucous origin, the slow infiltrative invasion by the neoplastic tissue, and the histological similarities between both tumor types declare their close relationship. The so-called carcinoma of the appendix is in reality only a carcinoid, and is not at all like the primary adenomatous, colloid forming, carcinoma found in the intestinal tract.

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[1] This case is shown here by permission of Dr. Bulkeley.

Cases of genuine primary adeno-carcinoma of the small intestine and appendix, have been described, but these tumors take their origin from the cells of the mucous membrane, and are histologically similar to corresponding tumors in the colon. They are of very rare occurrence, while the submucous carcinoids are relatively common.

A review of the literature shows that the carcinoids of the appendix may occur under a variety of forms. Most of them form a white neoplastic bulb at the tip; others occur as pinhead-sized nodules in the submucous layer. A few grow at the proximal end of the appendix, and others may grow in the middle. Sometimes the whole organ is involved. In the vast majority of cases, the tumor is confined to the appendix, and has been found only occasionally in the neighboring lymph nodes or in other viscera. Histologically, they present very much the same picture as shown by the tumor in Cases V and VI.

The carcinoids of the small intestine are usually accidental findings in old individuals who have come to autopsy from some other cause. These tumors are submucous in origin, and are situated on that side of the gut directly opposite the mesenteric attachment. They may occur as solitary nodules or as multiple primary neoplasms scattered through the intestine. The usual site is the lower end of the ileum, but they may be found in the duodenum, jejunum, and even the large intestine. These tumors show a very slow infiltrative growth, but metastatic tumors in the neighboring lymph nodes and other organs are rare. Under the microscope they present a picture quite similar to the neoplasms in Cases I, II, III and IV.

Many theories have been advanced to explain these carcinoids, but the ones most worthy of consideration have been advanced by Burckhardt and by Oberndorfer. Burckhardt believes that the tumor tissue takes its origin from the normal cells of the intestinal mucous membrane. The cells break off from the infiltrates the submucous layer very slowly. Some of the cases described by Burckhardt, show structures very similar to the deepest portion of the crypts of Lieberkühn, and the neoplasm intestinal crypts, and on the strength of this he interprets the carcinoid as a tumor of the intestinal mucosa analogous to the basal cell carcinomas of the skin, described by Krompecker.

Oberndorfer takes the view that the carcinoids arise from an embryonic inclusion of enteroblastic cells in the submucous layer of the intestine. The primary multiple occurrence in many cases, the submucous situation of the tumor, and its position on the side of the gut opposite the mesenteric attachment, support this theory, for as Oberndorfer points out, such undoubted congenital malformations of the digestive tract as adenomyomata, and aberrant pancreatic glands, show the same characteristics.

Of the two theories, the one advanced by Oberndorfer is the most plausible, though it does not clear up many points. It is very probable, however, that these carcinoids are small tumor formations derived from congenital inclusions of primitive enteroblastic epithelium. It is also conceivable that the snaring off might have taken place at different periods in the embryonic life of each individual case. The included cells would thus vary in their degree of differentiation which would, perhaps, explain some of the minor histological differences displayed by different carcinoids.

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Discussion:

Dr. Lambert: I saw one of these tumors last year situated in the duodenum in the subumucosa. In that one I thought that while the grouping of the cells in solid nests looked like carcinoma, the individual cells did not look like it. They were uniform in size, deeply staining, but did not give an impression of malignancy. I think carcinoid is a good term.

Dr. Wood: The sections are very interesting. The morphology is strikingly like carcinoma of the appendix. We have found the latter in 1 per cent. of cases. Some specimens have tumors of very small extent, but in others the growth involves a very large portion of the lumen and tip. In one case the middle of the appendix was involved. Mitoses are never seen in the cells. The growths seem to be quiescent. I think carcinoids is a good enough name. Still, we know that a basal cell or even a squamous cell epithelioma may stay quietly in the skin for a number of years and then when something starts it up, may become clinically malignant.

HYPOPLASIA OF THE ADRENALS IN ANENCEPHALY

ALWIN M. PAPPENHEIMER

(*From the Department of Pathology of the College of Physicians and Surgeons, New York City*)

The almost regular occurrence of hypoplasia of the adrenals in association with malformations of the brain has been known for many years. At the end of the eighteenth century and the beginning of the nineteenth, when the very minute description of monsters was a favorite pursuit, this association was repeatedly observed. The first observation is attributed by the elder Meckel to Morgagni. In the older references one finds the statement frequently made, that the adrenals in these cases of anencephaly and kindred malformations may be entirely wanting. More recent observers, however, are inclined to be sceptical as to their complete absence. R. Meyer, for example, in studying a large series invariably found the adrenals present, although in some cases the hypoplasia was extreme.

It is natural that much speculation should have arisen as to the cause of this almost constant association. The many theories have, however, led to nothing definite or even plausible. For example, Ilberg has suggested that the failure of the brain and cranial vault to develop may be due to the loss of the hemodynamic action of the adrenal during fœtal life. Were refutation of this idea necessary it would be found in the fact that in many, if not all of the cases, the medullary portion of the gland is present in normal or even excessive amount, and shows no qualitative alterations (Weigert, R. Meyer, Landau and others). Elliott and Armour have, indeed, studied the entire chromaffin tissue in one case, and have found it normally abundant.

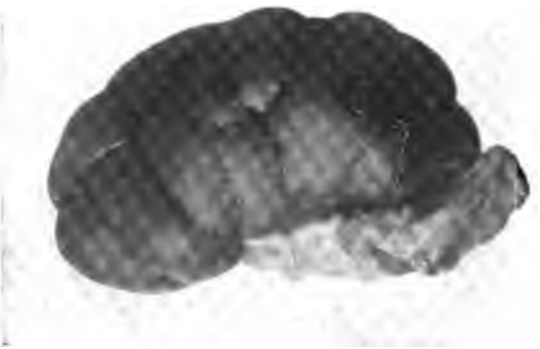

FIG. 1. Adrenal and kidney. Natural size.

A new point of interest has arisen in connection with the recent observations of Erwin Thomas, Elliott and Armour, Kern, Landau, Lewis and Pappenheimer and others that a large portion of the adrenal cortex which is so massively developed in the newborn infant is subsequently destined to degenerate. Within the first few weeks of post-natal life the cells composing roughly the inner two thirds of the fœtal cortex disintegrate and gradually disappear, leaving a collapsed vascular framework. We have, therefore, in this portion of the adrenal cortex a large mass of tissue which is functionally significant only in the fœtus. As to what that function may be we are, of course, totally ignorant.

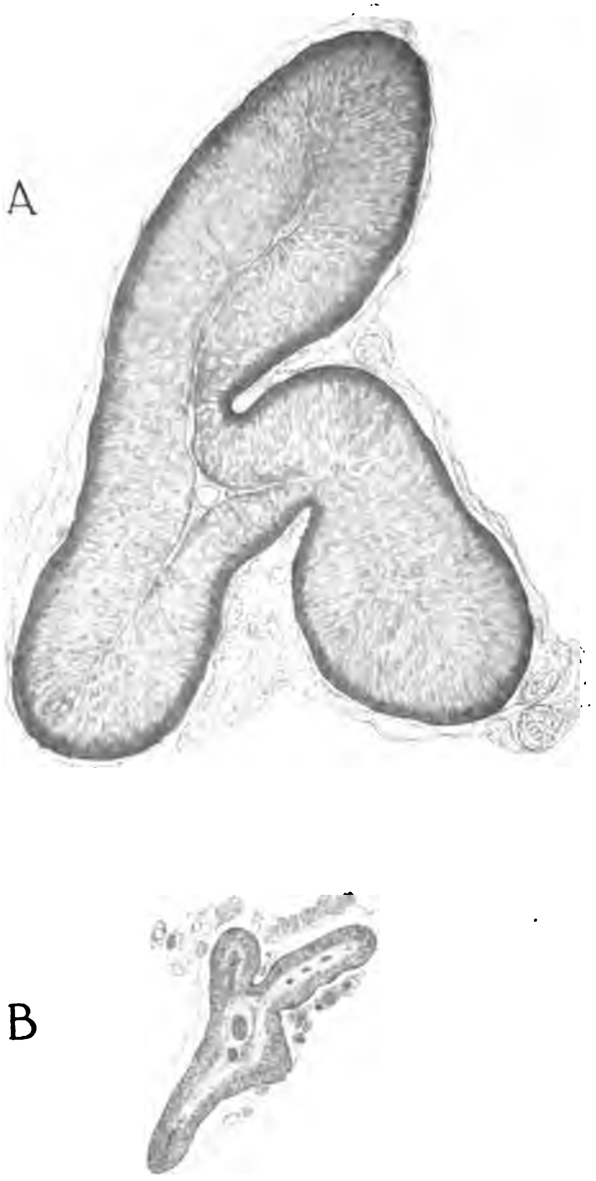

FIG. 2. Section through Adrenal: (*a*) of Full-term Newborn Infant; (*b*) of Anencephalic Infant. Magnification approximately × 10.

It is, however, of great interest to study the behavior of this fœtal zone of the cortex in the hypoplastic adrenals of anencephaly. In the case presented, and in the cases studied by Elliott, Armour and R. Meyer, it is very clear that the reduction in the size of the gland is due, almost wholly, to the diminished size of this fœtal zone, which is normally doomed to destruction in postnatal life. The zona glomerulosa and the outer portion of the zona radiata—the permanent fraction of the cortex—are normally laid down and have a rich lipoid content in part composed of anisotropic fat. The normally broad inner cortical zone, on the other hand, is represented only by three or four layers of irregularly disposed cells, easily recognized from the outer cortex by their low lipoid content and sharply marked off also from the medullary tissue in the central portion of the gland. The question arises, then, whether in these cases there may not take place in fœtal life an involution of this inner cortical tissue, which in normal infants is deferred to extra-uterine life. This might

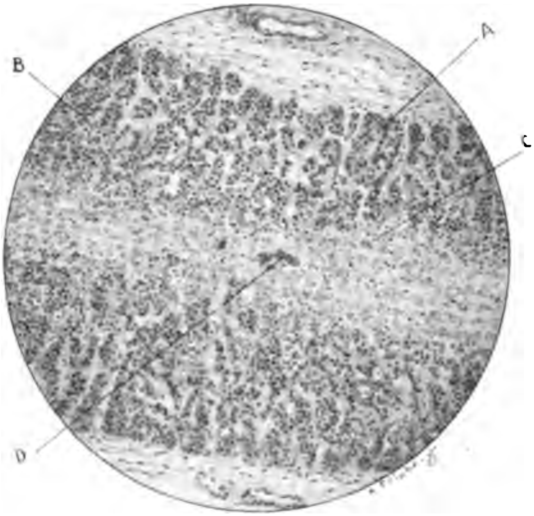

FIG. 3. Section through cortex of adrenal of anencephalic infant. (*a*) Glomerular zone; (*b*) fascicular zone; (*c*) rudimentary fœtal zone; (*d*) nests of chromaffin cells.

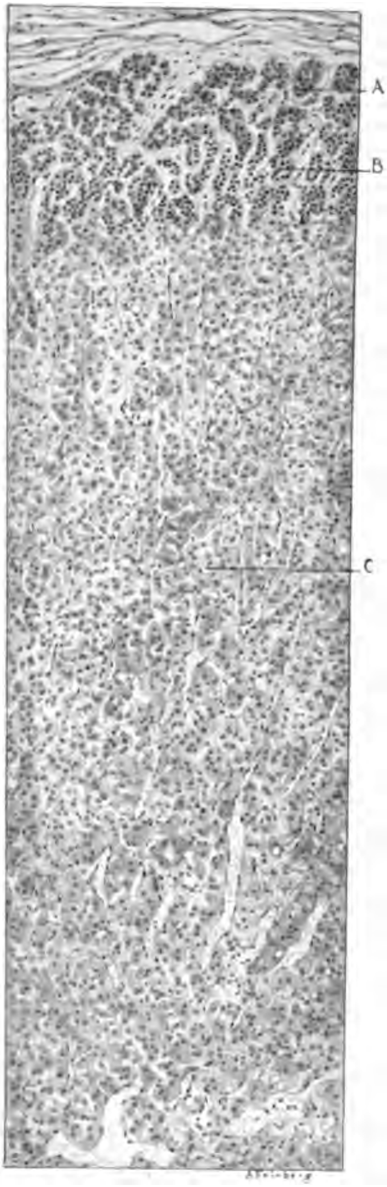

FIG. 4. Section through cortex of adrenal of normal newborn infant. Same magnification as Fig. 3. (*a*) Glomerular zone; (*b*) fascicular zone; (*c*) fœtal zone. No medullary tissue appears in the section.

have seemed probable from the observations which Meyer made upon the adrenals of two anencephalic fœtuses of two and five months in which the adrenal cortex in comparison with that of normal fœtuses of similar age was quantitatively and qualitatively normal. Landau has considered this point, but expresses himself very ambiguously in regard to it, saying in one place that he had never failed to find a "degeneration zone" present in these cases, but in other connections referring to the condition as a true hypoplasia.

From a study of my own case I am inclined to the view that this portion of the cortex has failed to develop rather than that it has undergone a premature degeneration. The individual cells of the thin layer which represents the fœtal zone, are all well preserved. The marked vascularity and the capillary extravasations which accompany the physiological involution of the cortex are wanting in this adrenal, nor does one find the cortical cells delimited from the abundant chromaffin tissue by a fibrous zone, such as ultimately results from the disappearance of the cells and the collapse of the fibro-vascular framework. Blood pigment in this case also is absent, although it is present always in the degenerating post-natal cortex.

The conclusion, therefore, which may be drawn from a study of this case, and from which it would be unwise to generalize, is that the small size of the adrenal is due wholly to the failure of development of the fœtal zone of the cortex; and that the permanent portion of the cortex, as well as the medullary tissue, is present in normal amount. This would suggest that it is this transitory cortical tissue which is in some way correlated with the normal development of the brain.

CANCER OF THE STOMACH IN A MOUSE

SHIGEMITSU ITAMI, M.D.

(From Columbia University, George Crocker Special Research Fund, New York, F. C. Wood, Director)

So far as can be ascertained no tumor of the stomach has been reported in a mouse since the two described by Murray.[1]

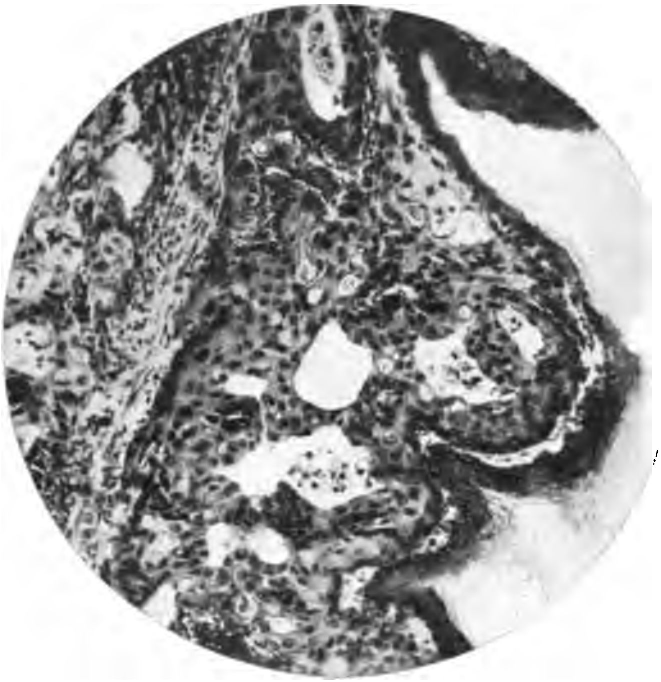

FIG. I. Primary squamous-cell carcinoma of the cardiac portion of the stomach of a mouse.

The mouse about to be described was a white female of unknown age, weighing 24.9 gm. and bearing two adenocarcinomata in the right axilla. These were extirpated on June 5, 1916, and did not recur.

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[1] Murray, J. A., Third Sci. Rep., Imperial Cancer Research Fund, London, 1908, p. 69.

In August, it was noticed that the mouse was not eating, and that its weight was decreasing; thus, on August 12 the weight was 25.0 gm., on August 19, 20.1 gm., and on August 26, 19.1 gm. On the 28th of this month, therefore, the animal was killed, and autopsied under aseptic conditions.

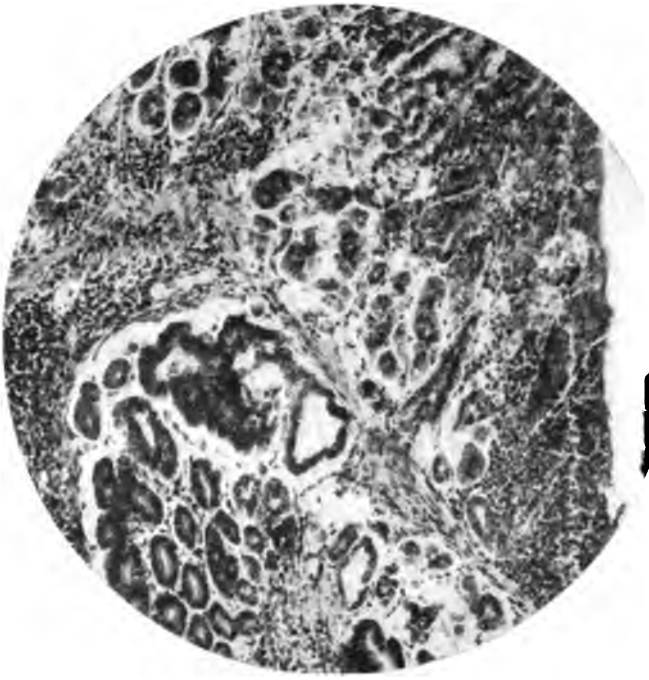

FIG. 2. Invasion of the deeper layers of the stomach by glandular structures, due apparently to irritation caused by the presence of the tumor.

The stomach was found much thickened along the greater curvature, the thickening being diffused over its squamous or cardiac portion. The glandular or pyloric third was not involved. The stomach was not adherent to the adjacent organs, and, except for collapse of the intestine, the other structures of the body appeared to be normal. No macroscopic parasite could be found, nor was any discoverable in the serial sections later examined. A frozen section showed the presence of squamous-cell carcinoma.

A fragment of the tumor was thoroughly washed in normal saline solution and transplanted into forty-eight mice; a larger series was not inoculated because it could be reasonably expected that only the exterior of the growth would be free of microorganisms, and not much of this could be removed without opening the stomach, a consequence which it was desirable to avoid. Up to the present time no daughter tumor has appeared, though a number of abscesses have developed, as was to be feared.

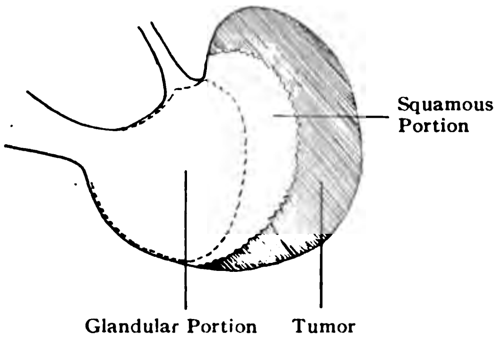

FIG. 3. Tumor of the stomach of a mouse. (Twice natural size.)

Serial paraffin sections, cut longitudinally along the greater curvature, showed a squamous-cell carcinoma situated near the pyloric portion and extending laterally under, and distinctly raising, the remaining normal squamous epithelium. The lower surface of the growth invaded the entire muscular wall, its downward and lateral spread producing an appearance identical with that described by Murray. The tumor was sharply demarcated from the glandular portion of the stomach.

Sections made from the other organs, including the lymph-nodes, revealed no metastases.

Although this mouse had two carcinomata in the mammary region, it is probable that the gastric tumor was not a metastasis. On the contrary, it is much more likely that it arose primarily in the squamous epithelium of the stomach; at any rate, it is directly continuous with this layer, which it closely resembles, and, furthermore, both axillary tumors were purely glandular, containing no suggestion of keratinization.

CARTILAGINOUS AND BONY NODULES IN THE TONSILS

EDWARD G. CARY, M.D.

(From the Pathological Department of St. Luke's Hospital, F. C. Wood, Director)

The occasional occurrence of cartilage in the tonsil has long been known, but it was after Orth's description[1] of two cases of cartilaginous and bony nodules in the tonsil that active interest in the phenomenon developed. Two years after Orth's publication, Deichert[2] reported two new cases in adults and also Orth's second case, a child of two years. Both these authors believed the nodules originated in misplaced embryonal rests of cartilage, coming from the second branchial arch. Deichert summed up the cases in the words, "We have cartilage and bone lying symmetrically in the deep connective tissue of both tonsils in individuals of various ages without especial recognizable pathological changes in the follicular apparatus." In one of his cases, a woman of thirty-five, the presence of a bony nodule 2 cm. in length in the stylohyoid ligament, a long styloid process, and enlarged lesser horn of the hyoid, existing together with cartilage and bone in the tonsil, strengthened his belief in the misplacement idea since all these structures come from the second arch. Furthermore, the nodules were supplied with a well-formed perichondrium and periosteum, and did not show the intimate relation to the surrounding connective tissue that might be expected in cases of metaplasia. The cartilage was hyaline in type and in the child was greatly in excess of bone—bony nodules of a few cells were found. The adults showed a preponderance of bone, formed, he thought, by metaplasia of the cartilage.

Walsham's[3] two cases were adults. One of these showed a preponderance of bony nodules, the other cartilage, and the latter was scattered through the adenoid tissue as well as the capsule and trabeculæ. The bilateral position and absence of hypertrophic changes he thought argued for embryonic origin. Wingrave[4] agreed with Walsham and Deichert that the cartilaginous nodules might well be of vestigial origin, but because of the multiplicity of the deposits and the frequency of association with morbid changes he felt that the bony nodules were metaplastic.

Töpfer[5] described the transition of connective tissue into cartilage and insisted that the nodules were metaplastic. Pollack[6] also strongly favored the theory of metaplasia for the following reasons: (1) Inflammatory processes were found in all his cases; (2) the bone occurred in portions showing active inflammation or evidences of inflammation in the past, and was in intimate relation to the connective tissue; (3) cartilage was absent in two cases, and in the third there was bony cartilage, indicating that cartilage is only an intermediate stage; especially since in other areas in the same tonsil the bone had plainly come from the connective tissue. Nösske[7] thought it not improbable that the connective tissue of the tonsil had a special ability for cartilage and bone formation due to developmental characteristics without making an abnormal displacement responsible.

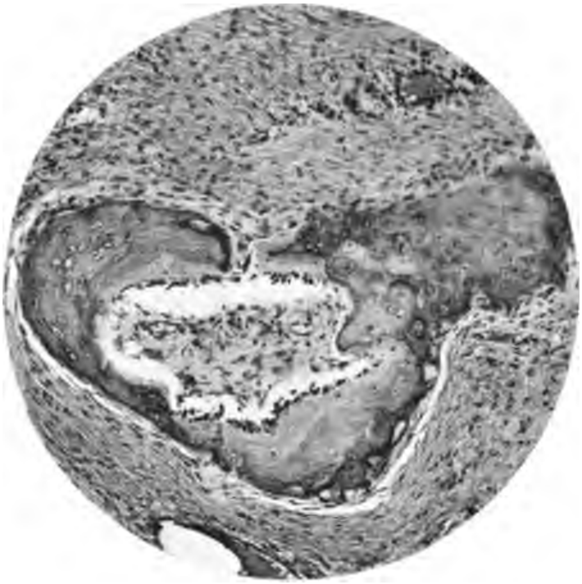

FIG. 1. Bony nodüle with bone marrow.

Reitmann[8] in a series of fifty cases, including embryos, infants and adults, found cartilage in the tonsil in 34 per cent. Ruckert[9] found about the same percentage in infants and stated that in all his cases the cartilage was embryonal in character. In a series of 412 cases of all ages from the embryo to advanced old age Lubarsch[10] found the deposits in 16 per cent. Most of these cases were tubercular patients and showed either tubercular lesions of the tonsils or tuberculosis in some other part of the body. The majority, though not all, of Lubarsch's cases showed inflammatory changes about the deposits, and this writer concluded that they arose both from inclusions and from metaplasia of the connective tissue in response to inflammation.

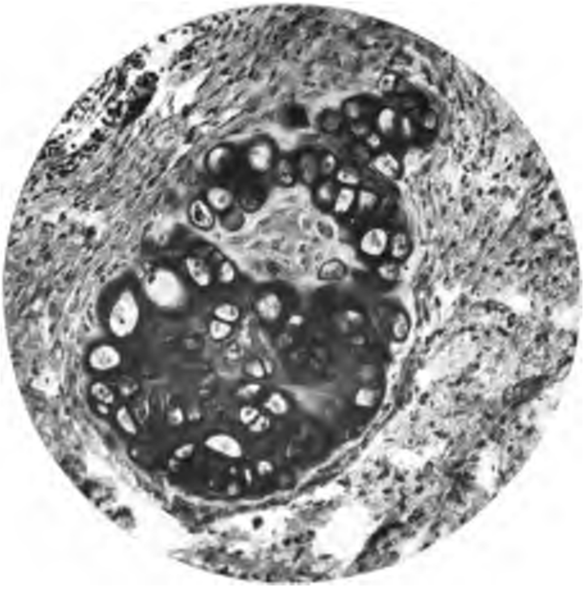

FIG. 2. Cartilaginous nodule with bone in the central portion.

The arguments advanced against the embryonal theory (Töpfer,[5] Pollack,[6] Nösske,[7] Lubarsch[10]) were: (1) The finding of cartilage only in the connective tissue portion of the tonsil and not in the adenoid or follicular part. Walsham[3] and Wingrave,[4] however, reported cartilage in the adenoid tissue. (2) The appearance of bone and cartilage only in inflamed regions. Deichert,[2] Walsham,[3] Wingrave,[4] Theodore[11] and Grosvenor,[12] however, reported cases in which inflammatory changes were not evident. (3) The observation of continuity of the nodules with bordering connective tissue. On the other hand, Deichert, Walsham, Wingrave, and Theodore describe a definite perichondrium and periosteum surrounding the nodules. (4) The absence of such nodules in children. Nevertheless, Reitmann, Rückert, Lubarsch and Grünwald[13] have demonstrated cartilaginous nodules in embryos, newborn and infants. The bilateral appearance of the deposits was made much of by supporters of the embryonal misplacement theory; but Lubarsch reported unilateral nodules and in these cases the inflammatory process was either present only on the side of the nodules or was most marked on that side.

Neither the metaplastic nor the embryonal theory of origin seems to cover all the cases. Ziegler[14] offers the explanation that besides the fœtal rests, such nodules may develop also in response to inflammation; because, in consequence of the embryonal relation of the tonsillar connective tissue to the branchial arch, it has an especial chondro- and osteoplastic aptitude. Grünwald[15] asserts that the rests come from the fourth, third and second arches, that they ordinarily regress, but that there may be primordial cartilage as well as chondrogenic cells left behind.

The case I have to report is that of a woman of thirty who gave a history of recurrent attacks of tonsillitis. On the cut surface of the tonsils after they were removed could be made out many small gritty spicules that in the microscopic section proved to be cartilaginous and bony structures embedded in the dense capsule of the tonsil and also appearing in the thickened cortical trabeculæ. As evidence of an inflammatory reaction there was swelling of the follicles, partial replacement of the adenoid tissue by connective tissue, infiltration of the cortical trabeculæ by small round cells and plasma cells, and near the crypts were frequent polymorphonuclear leucocytes. In the capsule near the base, the cartilaginous and bony nodules were particularly plentiful. Some of these nodules contained both cartilage and bone, some only cartilage, and a few bone that was not well separated from the surrounding connective tissue. While for the most part the nodules consisting of both bone and cartilage are made up of a central portion of cartilage surrounded by a ring of bone, the reverse condition occurred and the bone was deposited in the central part of a cartilaginous nodule. In places the bone showed a central cavity in which marrow had been formed; and these nodules contained cells corresponding in morphology and position to osteoclasts and osteoblasts. For the most part the bone nodules were supplied with a definite periosteal covering. The cartilage showed a gradual transition to a spindle type of cell and a blending of these with the surrounding connective tissue. The cartilage was hyaline in type with the usual vacuolated cells with reticulated protoplasm, oval and round nuclei, and definite well-formed matrix.

The only point of variance in the above case with those described before is the well-developed central bony deposit surrounded by cartilage. Theodore[11] mentioned as an argument against the inflammatory origin of these nodules the fact that the youngest cartilage cells occurred in the central part of the nodules and that the bony deposits were outside. In the case presented the reverse condition was found.

The theory that best explains the origin of cartilaginous and bony nodules in the tonsil and meets the conditions described by various writers is that, as Ziegler expressed it, we have embryonal rests and an especial chondro- and osteoplastic aptitude of the connective tissue cells in this region.

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Discussion:

Dr. Wood: It is interesting to see how frequently cartilage occurs in the tonsil. I do not think the theory of metaplasia explains the presence of hyaline cartilage. Everybody has tonsillar inflammation to a certain extent, and I doubt that chronic inflammation alone will cause the formation of cartilage. Cartilage occurs near or in the salivary glands, or in regions where, in the embryo, cartilaginous tissue is abundant; so that it has always seemed to me possible that cartilage cells may be left in the tissues and that these may grow if stimulated. Tumors of cartilage occur in the complex regions about the submaxillary and parotid glands and in the region of the bronchial arches where much cartilage tissue disappears early in embryonic life. Some of the cells perhaps remain and start growing later. Formation of bone marrow is common enough where bone occurs. In the aorta and kidney it is found, but I have never seen cartilage formation there.

Dr. MacCallum: I think one ordinarily sees the heteropic development of bone in those places in which necrotic tissue has been preserved for a long time, surrounded by living tissue. There marrow cavities are formed and the bone is laid down by osteoblasts. I have, however, recently seen a similar case in which cartilage was found in considerable quantities in the tonsil.

Dr. Pappenheimer: I have seen islands of cartilage in simple cystic kidney.

Dr. Wood: One might expect that cartilage would be found in congenital cystic kidneys, but my experience is that of Dr. MacCallum. Bone is not infrequently seen in the ovary and in many other sites, but I have never heard of the formation of cartilage in a lymph node, though bone develops often in old necrotic areas in nodes.

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A CASE OF LINITIS PLASTICA

ELI MOSCHCOWITZ, M.D.

The specimen presented is a typical case of linitis plastica, due to scirrhous carcinoma of the submucous coat of the stomach. It is of especial interest because there are no visible changes in the gastric mucosa.

The patient, a man 70 years old, was admitted to Beth Israel Hospital, April 18, 1916. He complained of alternating constipation and diarrhea of three weeks' duration. He had suffered from dull pains in the epigastrium, which were aggravated by food and relieved when the stomach was empty. For the last three days he had vomited all food. He had lost about ten pounds. Physical examination revealed a hard mass deep in the right hypochondriac region, but was otherwise negative. The patient died two weeks after admission. At autopsy the following anatomical diagnosis was made: Linitis plastica; chronic diffuse nephritis; dilatation and hypertrophy of the left ventricle; brown atrophy of the heart muscle; chronic congestion of the viscera.

The stomach was of the typical leather bottle type and unusually small, measuring 12 cm. in the transverse diameter and 8 cm. in the vertical. The peritoneal surface was smooth. The wall of the stomach was about 1.25 cm. in thickness and fairly uniform throughout. It was very firm and on cut section the submucosa was unusually broad. The mucosa appeared intact throughout revealing not the slightest ulceration or tumor anywhere. It was pale red and covered with a small amount of mucus. The thickening of the stomach wall extended to the first part of the pylorus. There were no enlarged glands.

Numerous microscopic sections taken from various portions of the stomach showed a scirrhous carcinoma involving the submucosa and extending into the muscular coats. No point of origin from the mucous membrane was found. The mucosa showed no infiltration of the growth whatever. There was however no other conceivable origin for the tumor and if sections were taken from many more areas in the stomach wall the primary growth might be found.

PRIMARY ALEUKEMIC SPLENOMEGALY—BANTI'S DISEASE

(SUMMARY)

ELI MOSCHCOWITZ, M.D.

The specimens presented are three spleens; the first two from patients in whom the diagnosis of Banti's disease was made; the third, from a case of splenomegaly associated with polycythemia.

CASE I. The patient was a married woman of sixty. For a year she had noticed a steadily growing weakness, and more recently a slight jaundice and swelling of the ankles and feet.

On physical examination she was found emaciated, and profoundly anemic. There were signs of an old tuberculosis at both apices. The liver was slightly enlarged. The spleen extended to the umbilicus. Its edge was irregular and hard. The abdomen contained fluid. The hemoglobin was 35 per cent. and fell to 10–15 per cent. a day or two before death. The red blood cell count was 2,850,000, gradually diminishing to 1,200,000; the leucocyte count 7,000, of which 78 per cent. were polymorphonuclears and 22 per cent. lymphocytes. She died from anemia.

At autopsy the anatomical diagnosis was: Emphysema and healed tuberculosis of the lungs; chronic pleurisy with adhesions, hypertrophy and dilatation of the left ventricle, atheroma of aorta; angioma of the liver, chronic diffuse nephritis and splenomegaly.

The spleen measured 24 × 20 × 15 cm. and weighed 1,165 gm. after fixation. It was densely adherent to the costal wall. The capsule showed the *Zuckerguss* appearance. The pulp was firm but easily indentable, and was deep reddish purple. The connective tissue was enormously increased and the trabeculæ prominent. The Malpighian corpuscles were not visible.

On microscopic examination the spleen showed marked fibrosis, seen as large masses of cellular fibrous tissue which obliterated the sinuses and compressed the veins. The trabeculæ were enormously thickened. The Malpighian bodies were few in number and small and some showed a fibrosis extending out from the central vessel. The pulp was diminished in cellular content and deeply engorged with red blood cells. Many of the vessels in the trabeculæ showed thickened walls. The sinuses were normal in size. The splenic veins in the pedicle showed a marked endophlebitis with plaques of lime and bone in the walls. Sections of both spleen and vessels were stained by the method of Levaditi and no treponema pallidum was found. The liver showed no cirrhosis.

Case II. The second specimen is from a woman forty-seven years old who gave a history of hemorrhages from the mouth and bowel. She complained also of weakness and loss of weight. These symptoms had lasted five weeks. She had suffered a similar attack twelve years before. When admitted to the hospital the patient was pale and emaciated. Her spleen, which extended to umbilicus, was hard and very irregular. The liver was not enlarged. There was ascites and nine to ten liters of fluid were repeatedly removed. Examination of the blood showed: hemoglobin, 50 per cent.; red blood cells 3,460,000; white blood cells, 14,200, of which 85 per cent. were polymorphonuclears, 12 per cent. lymphocytes, and 3 per cent. eosinophiles.

An autopsy revealed healed tuberculosis of the bronchial lymph nodes, brown atrophy of heart, atheroma of aorta and cardiac valves, chronic parenchymatous nephritis, chronic peritonitis, and splenomegaly with fibrosis.

The spleen measured 25 × 15 × 8 cm. and weighed 8 lbs., 4 oz. The surface was very irregular, with numerous deep depressions to which omentum was adherent. At the upper portion, a mass of splenic tissue, the size of an orange, had been almost severed from the remainder of the spleen by a deep fissure. The capsule was much thickened and covered by numerous hard irregular white plaques. The pulp was firm and brownish red. The trabeculæ were much thickened, the Malpighian bodies were not visible. The organ contained numerous fresh, and healed, infarcts. The splenic vessels in the pedicle are much thickened and calcareous.

Microscopically there was extreme fibrosis of the spleen, which contained very little of the cellular pulp. The fibrous tissue had in part undergone hyaline degeneration. The splenic veins were normal in size. The Malpighian bodies were small and showed fibrosis; the central arteries were thickened and frequently hyaline. The infarcts showed deposits of lime and bone. The splenic veins in pedicle showed profound endophlebitis and calcification. Sections stained by Levaditi showed no treponema pallidum.

The liver was swollen and there was some fatty degeneration but no cirrhosis.

Case III. This was a case of splenomegaly associated with polycythemia in a man of 52 who had suffered from angina pectoris. For one year he had had alternating constipation and diarrhea, occasionally with blood in stools. He had lost weight and strength.

Physical examination showed the heart enlarged, the liver slightly enlarged and an enormous hard spleen reaching to the umbilicus. There was no ascites. The blood count was as follows: Red blood cells, 6,700,000; hemoglobin, 100 per cent.; leucocytes, 15,000; polymorphonuclears, 80 per cent.; lymphocytes, 20 per cent. There was no fever. A splenectomy was performed, and the patient died the following day.

The anatomical diagnosis made at autopsy was: Fresh lobular pneumonia; hypertrophy and dilatation of left ventricle; dilatation of right ventricle; chronic congestion of liver and kidneys; fat necrosis of pancreas; and recent thrombosis of the splenic veins.

The spleen measured 20 × 11 × 9.5 cm. and weighed 1,190 grams. The surface was smooth except in the upper portion where there were adhesions. The organ was hard and reddish brown, and the trabeculæ were increased in size and number. Scattered throughout were numerous stellate hemorrhagic areas, conforming in outline to some of the large trabeculæ.

On microscopic examination the spleen showed a thickened capsule and marked fibrosis, the pulp being almost entirely replaced by connective tissue. The pulp cells were few in number; the splenic sinuses moderately dilated. The Malpighian bodies were reduced in size and in number. There was considerable extravasation of blood in and around some of the trabeculæ. The blood vessels in the trabeculæ showed slight thickening. In the splenic veins of pedicle there was slight endophlebitis, and in some a recent, but pre-operative, thrombosis. Sections stained by Levaditi showed no treponema pallidum. The liver showed chronic congestion with no cirrhosis.

These cases when grouped clinically fell into two classes: Cases I and II, Banti's disease; and Case III, splenomegaly associated with polycythemia.

The pathological findings common to all are the following: (1) Splenomegaly. (2) Diffuse fibrosis of the spleen. (3) A thickened and partly adherent capsule. (4) A notable diminution in size and number of the Malpighian follicles—a change in many instances due to fibrosis proceeding from the central artery. (5) An endophlebitis of the splenic veins. (6) A liver free from cirrhosis.

The characteristic clinical features of Banti's disease are the following: (1) Absence of known etiology; (2) a period of splenomegaly and anemia; (3) a transitional period in which swelling of the liver is prominent; (4) a final period in which the liver is small and there is ascites; and (5) a fatal termination by hemorrhage or anemia.

Pathologically, Banti claims the following findings as characteristic of his disease: (1) A marked fibrosis of the reticulum. (2) Fibrosis of Malpighian follicles, extending from the artery, which often shows hyaline degeneration. These changes he terms "fibroadenie," because the glandular structure of the organ is preserved. The fibrosis is not of inflammatory origin, he holds, because no fibroblasts are found in such spleens—in other words, it is a primary sclerosis. (3) A cirrhosis of the liver differing in no way from the Laennec type. (4) In many cases an endophlebitis and calcification of the splenic veins. (5) Freedom from general glandular enlargement. Banti explains the pathogenesis on the theory that the splenomegaly has an infectious origin, that the enlargement of the spleen produces a toxin which in turn causes the cirrhosis of the liver and the changes in the splenic veins, and that the anemia is due to the toxemia and to the hemorrhages.

Reviewing the clinical and pathological features of Banti's disease, it is found that there is no single feature or group of features that are pathognomonic. The criteria propounded by Banti himself do not hold on strict analysis. For instance, perfectly characteristic cases of Banti's disease have been reported for which a definite cause such as syphilis or malaria has been determined. The various stages which Banti describes also have caused frequent confusion. There have been many cases of Banti's disease (including the two reported) which showed no cirrhosis, although clinically they were in the third stage of the disease. The pathological characteristics described by Banti have been found in other conditions. Indeed practically all authorities agree that a diagnosis of Banti's disease cannot be made on a basis of anatomical findings.

Banti's theory of the origin of the disease is also unsatisfactory. It is inconceivable that a toxin should cause a splenomegaly which again causes a toxin which produces a cirrhosis in another organ. Moreover there is no reason to assume that the fibrosis is not of an inflammatory origin.

Banti believed he found confirmation of his hypothesis that the other changes were secondary to the splenic lesion in the supposed cure of the disease by splenectomy. However from a study of so-called recoveries one is hardly justified in regarding splenectomy as a cure for Banti's disease. In some cases results have been reported too soon, in others there is doubt as to whether the disease was actually that described by Banti.

A study of these three cases and of the published reports on this disease leads to the following conclusions:

1. The complex known as Banti's disease is entirely a nosological entity. It has no well-defined characteristics, either clinical or pathological.
2. Banti's disease is identical with the "splenic anemia" of Griessinger.
3. The value of splenectomy as a cure for Banti's disease must be open to question.

Discussion:

Dr. Wood: I remember one case operated on at the German Hospital—Dr. Willy Meyer would remember it. Six months later the patient showed a greatly improved condition and went back to work. This was a typical Banti's case with a very large spleen. There was a high degree of anemia which was evidently secondary, and the improvement was very interesting. At the time of operation there were two and one half million red cells and two months later the blood count was normal. Sections of that spleen are on record at the hospital. Many of these cases are very puzzling. In one recent case of splenic anemia, there was found a large gumma of the spleen. In another case of polycythemia there was a large tumor found. This was a fibrous spleen rather than one of type known as Banti's disease.

LEPROSY

W. G. MACCALLUM, M.D.

A number of lantern slides were exhibited illustrating various forms of leprosy seen in the Fiji Islands, Java, Bali, Singapore and Kuala Lumpur. The well-known histological characters of the lesions of the skin, the nerves, and the internal organs were discussed, and to some extent illustrated by the material derived from two cases which were studied at autopsy in Singapore.

Especial stress was laid upon the fact, which seems not to be mentioned in the literature, that the large vacuolated cells which form so conspicuous a feature of the lesions everywhere, and with which the bacteria are associated in such great numbers, are really cells loaded with fat globules. When stained with Sudan, the masses of tissue which form the bulk of the nodular thickenings of the skin are found to take a bright red stain, since all the cells, from the small mononuclear wandering cells through all stages to the very large foamy structures, contain in their vacuoles abundant globules of fat. Attempts to show that this fat is doubly refractive were unsuccessful with frozen sections of the formalin material, but it is possible that in the fresh state the globules may prove to be anisotropic. Further work is being undertaken to determine their exact nature. It seems possible that the fat may have some protective relation to the bacilli so that they can be reached best by fat soluble drugs.

Discussion:

Dr. Moschcowitz: I would like to ask Dr. MacCallum if he knows how frequently those who care for the lepers develop leprosy.

Dr. Ewing: I should like to ask what proportion of people in these islands have mild or unrecognized leprosy. Are there any data? Ever since the famous Early case, which was undoubtedly leprosy, I have felt that diagnosis in the early stages was difficult. I often wonder whether cases do not slip through our hands. If the early stages are not recognized it must be prevalent throughout the whole population. I wonder to what extent these cases are complicated with syphilis. Looking at these pictures I am reminded very forcibly of syphilitic lesions. I should like to know also to what extent tuberculosis is present among lepers.

Dr. MacCallum: In regard to Dr. Moschcowitz's question about the attendants, I did not wait long enough to see; but in a summary of the literature published in the first volume of the *Journal of Tropical Medicine*, very few instances are reported of people in contact with lepers contracting the disease. It appears in most cases that the contact is very intimate before infection takes place. You can't believe all the tales you hear in the East. You do hear of cases where slight contact was supposed to have caused infection, but you hear of attendants working a long time with lepers, and escaping scot free. Very few doctors or sisters of charity have been infected. One is struck at once by the apparent daring of the attendants. In the Singapore hospital they take no precautions whatever. One had been there seven years, and one nine years. At autopsy the attendants did handle body with cloths, but that was because of the disagreeable nature of the ulceration.

In answer to Dr. Ewing, I should suppose there were many mild cases but it was quite impossible for me to find that out. It would take long residence and intimate study in those countries. I saw many slight cases, recognizable only when one looked for them. The surest way of studying early cases is by studying the nasal secretions. The early lesions may be in the nasal septum. As to combination or confusion with syphilis, that seems plausible; but I think that the diagnosis of these cases in the pictures has been made on the finding of lepra bacilli. In Penang there is a large leper colony and the cases are diagnosed by bacterial study. They may have syphilis or tuberculosis as well. I had one autopsy where the patient was a leper and also tuberculous. In the Fiji Islands there is no syphilis, except in the indentured laborers who are Hindoos. In the Malay states this is not true. The syphilis observed there is beyond anything we have ever dreamed of. My first experience of leprosy was a case that had been diagnosed as syphilis by a distinguished dermatologist. She was supposed to have tuberculosis of the lungs but I am not sure it was tuberculosis. It was a very distinct leprous case.

TUMOR PRODUCTION IN ANIMALS

DUDLEY H. MORRIS, M.D.

(*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director*)

In spite of the wide divergence of current views on the etiology of cancer, one factor is universally admitted to be necessary in order that a transplanted tumor may grow in a mammalian host. The living cancer cell has come to be regarded as this essential unit in dealing with transplantable tumors. But that a living cancer cell is not the primary cause but merely the vehicle of cancer becomes evident when we consider the origin of the cancer cell itself. Practically all authorities are in accord, that the cancer cell develops from some ancestral normal body cell of homologous type. And this transition from the normal to the cancerous may take place through the influence of some agent, the nature of which is unknown, acting in such a manner as to produce a permanent alteration in the normal tissues acted upon. What then is this agent, and by what means can it be studied? Obviously the best way to investigate the properties of such an agent would be to isolate it, if possible, and obtain it free from the soil where it has lodged and by its action produced the cancerous change. While this isolation of a tumor-producing agent appears to have been accomplished in a few instances in chicken tumors, it has never been done with other cancers. Rous was able by means of filtration through Berkefeld filters impermeable to ordinary bacteria, to isolate a cell-free agent capable of transmitting a chicken sarcoma from one animal to another. Repeated attempts have failed to demonstrate that the filtrates of any existing mammalian tumor can cause such growth in another animal. It is not difficult to imagine why, unless the exact conditions favorable to growth were provided, these experiments should fail, since such variants as dosage, degree of dilution, length of observation, character of tumor, etc., might well have a profound effect on the outcome.

With these possibilities in mind, experiments were carried out on a large series of animals and a number of variations in method have been employed.

In these experiments eight sarcomata, six carcinomata, and one basal-cell epithelioma were used. In nearly all cases a small amount of kieselguhr (silicious or diatomaceous earth) was injected with the filtrate, in order to furnish what Rous had shown to be the best conditions for tumor growth in the chicken sarcoma he studied.

The series comprised in all about three thousand animals, approximately two thousand mice and a thousand rats being used.

About one half the mice and one third the rats survived for five months, and many of them had nodules at the site of inoculation at this time, but no tumors had developed. These nodules when removed showed the histology of rather cellular granulomata obviously developing about the kieselguhr which was injected with the filtrate. After eight months, about one third the mice and about seventy-five rats remained alive. All the animals were negative to tumor growth except four rats in one box. These were part of a series of fifty animals which had been injected with a filtrate of the Flexner rat carcinoma to which a very small amount of kieselguhr had been added. Of this series, six animals out of fifty remained alive, four in the box in question and two in another box. The six animals, including the four animals in the positive box, had had the following treatment. They were first splenectomized, and ten days later 1 c.c. of a Berkefeld filtrate of Flexner rat carcinoma (7 gm. tumor to 70 c.c. Ringer's solution) was injected into and beneath the skin of the left flank just below the costal margin. This site was chosen merely because it was the area which had been shaved for the preceding splenectomy. To the entire filtrate (60 c.c.) were added a few drops of a milky suspension of kieselguhr in Ringer's solution. All were young animals not over four months old when injected with the filtrate. After six months these animals had been negative, except for two small opaque white nodules at the site of injection of the filtrate. These nodules were common enough in the other animals and represented merely a reactive proliferation of the subcutaneous tissue to the filtrate-kieselguhr irritant. After eight months, however, one of these lumps, still white and opaque, had reached the size of 1 × 1 cm. and the other, though smaller, had taken on a lobulated appearance and distinctly bluish color. In the third rat, a nodule the size of a pinhead was present at the inoculation site. The fourth animal was negative. These animals henceforth were observed daily and the following events were seen to occur. The bluish nodule grew fairly rapidly and soon surpassed the opaque fibrous one in size. It was then removed, except for a small fragment, and the greater part was used for inoculation into seventy-two young rats, a slice being saved for microscopical examination. This tumor (Filtrate F. R. C. No. 1 [Fig. 1]), for such it proved to be, was an adenocarcinoma of a remarkable type, totally different from the Flexner rat carcinoma and, in fact, from any rat tumor in the laboratory.

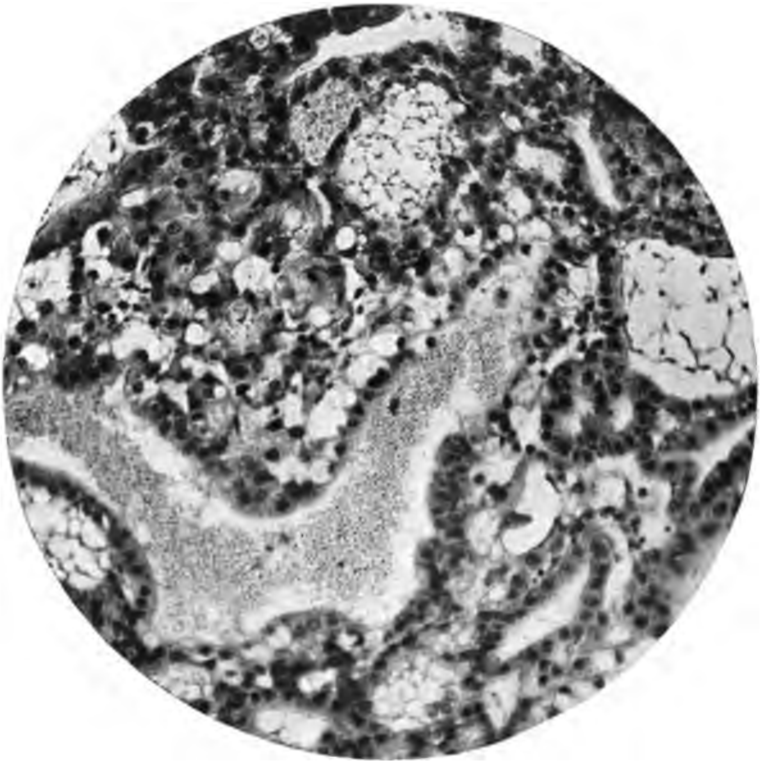

FIG. 1. Filtrate F. R. C. No. 1. (C. F. 10674). Typical adenomatous area, showing hemorrhagic character, vacuolization of cells, and production of mucus and colloid. × 200.

The microscopical sections of this tumor showed it to be a hemorrhagic cystadenocarcinoma with intracystic papillary outgrowths of cells and masses of cells apparently free in spaces engorged with blood. In many places the acini were very large and their lumina empty; in others they were filled with colloid or mucinous contents. The tumor cells were relatively large, in places pyramidal or polygonal with large vesicular nuclei and resembled the cells of a serous gland. In other areas the acini were lined with a single layer of cuboidal or columnar epithelium. Here and there were scattered cells which greatly resembled the goblet cells, giving the acini somewhat the appearance of intestinal crypts. The protoplasm of the tumor cells was characteristically vacuolated and produced large and small globules of what appeared to be a colloid or mucinous substance. Certain of the cells were so distended with this secretion that they were swollen and the nucleus flattened to a mere granule at the periphery. Such cells as this, together with large aggregations of the mucinous substance, gave the tumor a characteristically reticulated appearance. In places the adenomatous arrangement was lacking and the cells showed an invasive tendency. Irregular strands or groups of polygonal cells were here found infiltrating connective tissue somewhat in the manner of scirrhous carcinoma.

During the month which elapsed after the removal of this tumor, the opaque white nodule in the second rat (Filtrate F. R. C. No. 2 [Figs. 2 and 3]) very slowly and almost imperceptibly increased in size; its margins assumed a slightly bluish tinge, and two minute bluish subsidiary nodules appeared at the lower pole. The pinhead nodule observed in the third rat (Filtrate F. R. C. No. 3), however, increased rapidly until it was larger than that in the first. It was manifestly bluish and hemorrhagic, but not much lobulated. In the meantime, a tiny nodule had appeared in the fourth rat. This, while no bigger than a grain of barley, was obviously bluish; it enlarged very slowly.

Five weeks after the removal of the first tumor, the second was removed for transplantation. Histological examination showed it to be a hemorrhagic cystadenocarcinoma of a type somewhat resembling the first tumor, but not at all like the Flexner rat carcinoma. Scattered through the tumor were foci of hyaline connective tissue in which were fibrin clot and remains of the kieselguhr which had been injected with the filtrate. Careful search of serial sections failed to reveal any traces of normal breast tissue or ducts. This tumor differed from the first chiefly in the tendency for the acini to be composed of epithelium in many layers and in the more solid character of the cancerous growth.

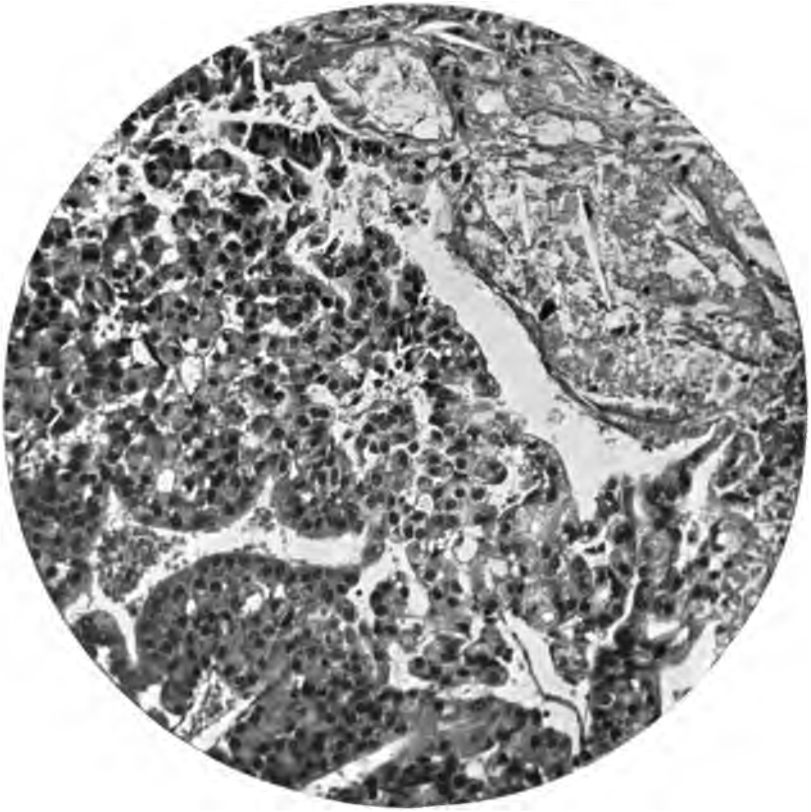

FIG. 2. Filtrate F. R. C. No. 2. (C. F. 10934.) Adenomatous area adjacent to kieselguhr remains, showing hemorrhagic character, vacuolization of cells, etc. × 200.

A few days after, the tumor of the third rat (Filtrate F. R. C. No. 3) was removed and transplanted. The sections of this showed it to be likewise a hemorrhagic adenocarcinoma, but of a type somewhat different from those previously removed. The acini were of two kinds, some similar to those previously described, others composed of masses of stratified columnar epithelium closely investing blood capillaries.

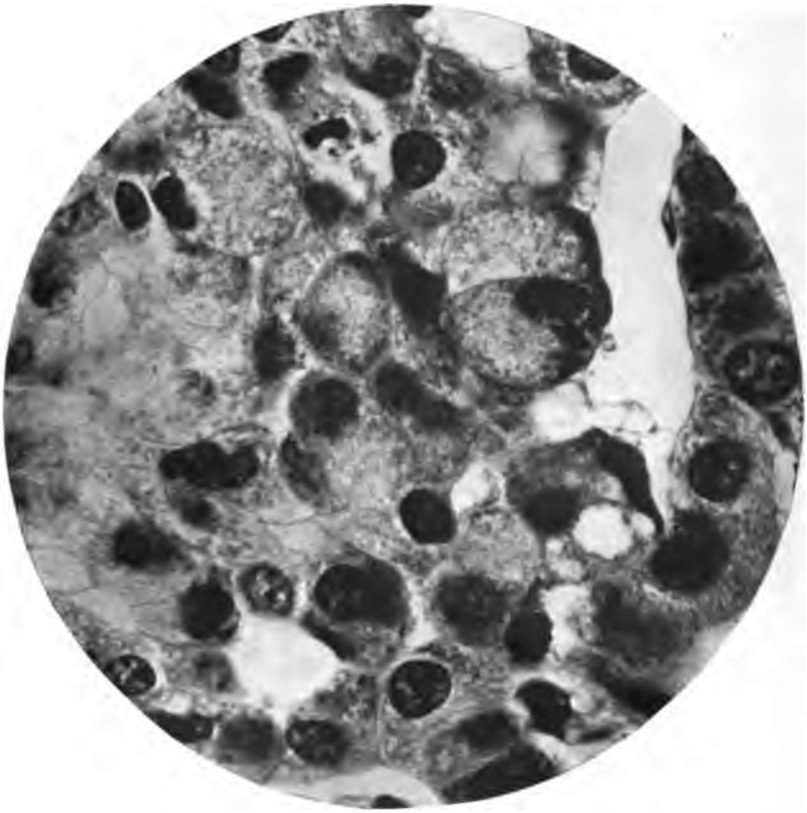

FIG. 3. Filtrate F. R. C. No. 2. (C. F. 10934.) Showing general cell characteristics, including vacuolization of cells and production of mucus. × 1,000.

The nodule in the fourth and last rat (Filtrate F. R. C. No. 4), while still very small, was removed 294 days after the injection of the filtrate. It measured roughly 4 × 5 mm., and was flattened and bluish. Serial sections showed it to be a hemorrhagic cystadenocarcinoma, resembling the first tumor. The cells were frequently vacuolated and there was abundant production of a mucin-like substance, which in places filled the lumina of the acini.

The transplants from the first rat tumor (Filtrate F. R. C. No. 1) became definitely palpable at the end of a month and grew very slowly in ten per cent. of the animals. At the end of nine weeks, the largest tumor had reached the size of about 8 mm. in diameter, when it was removed for transplantation. Microscopically it showed the characteristics of the cystadenomatous part of the original tumor (Fig. 4). All the tumors of this gen-

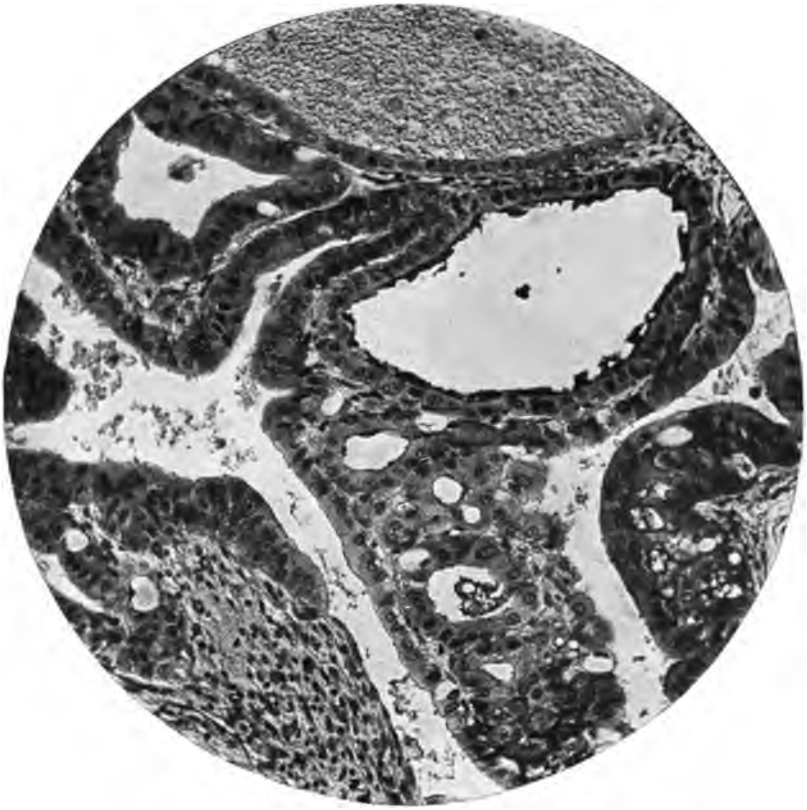

FIG. 4. Filtrate F. R. C. No. 1/A. (C. F. 10978.) Transplant of Filtrate F. R. C. No. 1. Showing preservation of hemorrhagic character, adenomatous arrangement, shape, size, staining characteristics, production of mucus and colloid. X 200.

eration preserved the hemorrhagic, nodular, and cystic character of the original tumor. It is yet too soon to observe the fate of the transplants from tumors No. 2, 3, and 4. The animal in which the second tumor arose has recently died. Autopsy showed pneumonic consolidation, but the remaining viscera showed nothing of pathological interest. There were no signs of metastases or other neoplastic growth. The spleen was absent.

There are six possible explanations of the results above recorded.

I. That through some mistake the animals had been inoculated with a transplantable tumor, either prior to or subsequent to the injection of the filtrate. This possibility may be eliminated for the following reasons:

1. The greatest care was taken in the preservation of the filtrate and use of the syringe to avoid contamination with the unfiltered tumor emulsion.

2. Each of the animals was indubitably marked by the removal of the spleen with the resulting scar.

3. The site of injection of the filtrate was the left flank, whereas the customary site for inoculation in the Crocker laboratory is the right axilla.

4. The morphology of the tumors differs from that of any rat tumors now being transplanted in the laboratory, and their hemorrhagic character is a feature rarely found in tumors that have been the subject of repeated transplantation.

II. That Flexner rat carcinoma cells have passed through the Berkefeld filter due to some imperfection in the mantle used. Against this possibility are the following points.

1. The filter was tested, before using with cultures of *B. prodigiosus* and, after using, with *B. megatherium*, and found to be impervious.

2. Red blood cells and leucocytes, both smaller than Flexner rat carcinoma cells, were found not to pass through the filter when this was tested with citrated blood. Erythrocytes, due to their flexibility in the fresh state, should be capable of passing through a very small hole should such exist. However, prolonged and

careful examination of the centrifugalized specimen failed to reveal any corpuscles whatsoever.

3. Filtrates of other tumors made with the same filters, both before and after Flexner rat carcinoma was used, failed in three thousand animals to produce tumors, which probably would have occurred had cells passed through. Many of these tumors had cells smaller than Flexner rat carcinoma cells, and some had a rate of increase and a growth adaptability greater than that of Flexner rat carcinoma.

4. The type of all the tumors found is totally different from that of the Flexner rat carcinoma. This tumor has been continuously observed in this laboratory for the past three years and so far as is known has not during that time presented an appearance comparable to the tumors found. Furthermore, the plates of the original tumor, as reported by Flexner,[1] fail to show any similarity to the present growths. It must then be assumed, on the basis of cells getting through, that, due to the exceedingly small dosage, or growth in kieselguhr granulation tissue, the tumor has changed its type from the definite regularity of the transplanted tumor to the bizarre irregularity and hemorrhagic character of the four tumors found. Furthermore, the cells must have changed in shape, size, and staining quality as well as in arrangement and must have acquired the secretion of mucus and colloid as a regular feature of their growth.

Lastly, the low percentage of takes and sluggish rate of growth of the transplants are not in the least like the Flexner rat carcinoma, which has had years of cultivation and can be depended upon to grow in nearly 100 per cent. of the stock rats, temporarily at least.

III. A third possibility to be considered is that four spontaneous tumors occurred in rats in the same box, and by chance at the site of inoculation of the filtrate. The frequency of breast carcinomata in white rats is not accurately known. McCoy's[2] statistics of 100,000 wild rats, in which complete autopsies were done, showed only 3 carcinomata of the subcutaneous tissue in the whole number. Bullock and Rohdenburg,[3] in a paper which has just gone to press, have found 2 carcinomata of the breast in 4,300 white rats. Woolley and Wherry[4] in 23,000 wild rats found not a single instance of carcinoma.

When we consider that here are four carcinomata all arising in animals in the same box and exactly at the site of inoculation of the filtrate, it will be seen how extremely improbable it is that these could have occurred fortuitously.

IV. A fourth possibility which must be considered is that due to irritation of the kieselguhr, and independent of the tumor filtrate, portions of breast tissue have been stirred to malignant growth.

As against this possibility it may be said that:

1. Kieselguhr as an irritant has been tried by numerous investigators in the attempt to cause malignant change, but without result. It may be urged, however, that sufficient time was not given for the transition to take place. Such may be the case, but I have tried kieselguhr and salt solution alone in about 500 of the 3,000 animals, both mice and rats, on the opposite side from which the kieselguhr and filtrate was injected. One hundred and fifty of these 500 animals survived for eight months and were completely negative to tumor growth; only in the four animals splenectomized and injected with the filtrate of Flexner rat carcinoma did tumors develop. At the time these tumors were found, six animals were the only ones alive that had been treated with Flexner rat carcinoma; of these, four developed tumors. Apparently the method would bear a causal relationship to the findings.

V. The fifth possibility, that splenectomy, by removing the resistance of the animal to spontaneous tumor growth, caused the tumors, is improbable, for other rats splenectomized and treated with kieselguhr and the filtrate of several other tumors failed to develop cancer, though they survived for eight months.

VI. The remaining possibility is that we are dealing with a filterable virus capable of stimulating cells of a new host to cancerous growth.

1. Against this it may be urged that many have tried with

uniformly negative results to find evidence of such a virus in mammals. It is hardly probable, however, that the exact conditions which were found to be successful in this case have been previously fulfilled, and, indeed, the time element alone would rule out most similar experiments.

2. The sarcomatous transformation of host connective tissue[5] about certain carcinoma grafts in mice is suggestive in this connection. This intimates that there is something in the cancer nodule which is contributed by the carcinoma cells and able to influence the cells of the host, causing them to become sarcomatous. Nevertheless, it must be recalled that sarcoma development occurs after about fifty-five days in the mouse, hence it is pertinent to inquire why six months was necessary to produce tumors in rats. It is possible that this discrepancy may represent simply a difference in concentration or individuality of the agents just as one tumor differs in its rate of growth from another. Moreover, it may be that epithelial cells require a longer time than connective tissue to be stimulated to malignant growth. Finally, this power is sometimes lost, though the carcinoma continues to proliferate.

3. We know of the existence of at least one such virus in a growth reported by Rous[6] as a malignant chicken sarcoma. While this is not epithelial in character, it is probably in the realm of malignant tissue transformation and this transformation is caused by a filterable virus.

In order to clear up and extend the results here recorded, further experiments are being carried out on a large scale, and these will be reported subsequently.

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6. ROUS, P.: *Jour. Exper. Med.*, 1910, xii, 696.

Discussion:

DR. EWING: I am very much impressed by the industry and care with which this work has been done. It is a distinct contribution. I am not able to agree with the statement that tumors in general are due to the action on normal cells of an external agent. I believe in the theory of cell autonomy. I think the doctor is assuming what he is trying to prove when he states that the source of the tumor process is external irritation. If, however, one starts out with this view he will feel inclined to interpret these results as Dr. Morris has done, as evidence of the artificial production of a tumor by a filterable agent. I think one would want to be careful about committing oneself to such a view. One should consider all possible sources of error as Dr. Morris has done in detail. In running over the possibilities which he mentions I would hesitate with one or two points and consider them worth further attention than he has given them. It is a well-known fact that workers who have injected various materials into various animals have encountered remarkable results, some of which have been interpreted as accidental and some in accordance with the opinion which the worker had in mind. I recall Lack's tumor from scrapings of ovarian epithelium in rabbits, and Schmidt's injections of mucor into guinea pigs. They got genuine tumors in their animals. Anyone who reads Schmidt's work must accept his conclusions if they accept his facts. Lubarsch injected embryonic cells into a guinea pig and the animal developed a remarkable tumor of the kidney. He regarded this as accidental. I don't think it is possible to set aside the possibility of pure accident in this series. It appears to me somewhat peculiar that this agent produced a different tumor from the Jobling strain. In regard to the work of Rous, a satisfactory explanation has not been given as to its true significance. I am inclined to think that if you grant all that Rous claims of a definite malignant tumor produced by a living virus, it would then be the height of folly to apply that demonstration to any other process in nature. It would be necessary to go over the whole field inch by inch and actually determine the fact if any other tumor yields the same results. It would be against all our knowledge of the wide diversity of pathological processes. All tumors are neoplastic reactions, but they are separated by hosts of factors relating to their special etiology. However, I am very pleased to have heard this contribution and I hope the doctor will be able to repeat his experiment.

DR. MACCALLUM: This paper is very interesting and it seems to me that if it is true that it is one of the most important things that has been done. I am inclined to agree with Dr. Ewing that it is necessary to do more work on this line before we can speak definitely. The injection of filtrate is an important thing and I am more settled in my mind in regard to the specificity of the tumors than Dr. Ewing says he is. It is an important point that the extract should have been injected and watched for eight months and then produced a different kind of tumor. That arouses more doubt in my mind than anything else. That very idea suggests another one, that all these tumors were in the same cage, and no animals in the other cages had tumors. Borrel observed frequently that all the animals in certain stages developed tumors and supposed them due to acarian parasites. Ehrlich ridiculed that idea, but some factor produced tumors in the one box and the others escaped. I don't believe Borrel carried out an exhaustive study on the subject, but in Paris some workers got whole colonies of animals suffering from this epidemic, as you might call it. I don't wish to throw cold water on this effort, I think it is only by such studies that we arrive at real knowledge. I hope Dr. Morris will be able to do this work over again and prove it is so.

Dr. Levin: So far as an explanation is concerned—I don't teach pathology, and I don't care about infective agents, specificity, or any other fixed idea—but I say that when we can produce a tumor the explanation can come afterwards. There is only one thing; I rise to make a motion that we demand another report of Dr. Morris whether or not this idea is proved. Borrel showed me his experiments in producing tumors in certain mice, but I heard nothing more about it. The greatest trouble we have with cancer research is that we never get to the second chapter, and that is the most important one. Success, it seems to me, always comes with the most peculiar tumor, thus we have the Rous tumor and the Flexner which was a sarcoma-carcinoma. This experiment is with 1,000 rats and the experiments should be continued on an even larger scale. Personally I would like to throw out the kieselguhr and I would not touch the spleen, but I do congratulate Dr. Morris on his results that he has obtained so far.

Dr. Wood: The spleen may not play a part at all, but it is a disturbing factor in the experiment. The logical thing to do is to repeat the experiments with and without splenectomies, and with and without the addition of kieselguhr. I think that in these cases the extract had nothing to do with the tumor production, but that a chronic inflammatory condition of the breast tissue was set up in the animals, such as occurs in chronic cystic mastitis or cystadenoma in human beings. Those tumors very closely resemble those conditions, and their morphology, in my opinion, indicates that they are primary growths. The Flexner-Jobling tumor used was derived from the seminal vesicles; and the tumors shown resemble those of the breast. It seems more probable that they arose spontaneously as a result of the irritation of the silicious earth injected rather than that the extract had anything to do with their appearance.

Dr. Hopkins: If this thing is a filterable virus, is it not conceivable that, if you transplant it into cells other than those in which it has been growing, it might produce a tumor of a new type, dependent on that of the cells in which it lodged? Ordinarily it is transplanted in tumor cells which are grafted on the inoculated animal. It is the grafted cells which multiply and consequently the new tumor is of the same type as the original.

Dr. Wood: If there is a virus, it would naturally stimulate local cells, not necessarily of the same type as the cells of the original tumor, so that it is more probable that a new tumor would be formed than that one of the original type would be reproduced. The Flexner-Jobling tumor is a well-adapted growth of a morphology quite different from that of the tumors shown by Dr. Morris.

Dr. Morris: I realize that it is in the nature of an attack on established opinion, to produce anything of this sort. I have every intention of carrying out this work on a large scale and sifting it to the bottom.

CONGENITAL ATRESIA OF THE ESOPHAGUS AND EXTENSIVE CONGENITAL BILATERAL HYDROCELE OF THE NECK

G. L. ROHDENBURG, M.D.

(*From the Pathological Laboratory of Lincoln Hospital*)

The two specimens of congenital malformation presented here are of well-recognized types, though neither is of frequent occurrence. The history of the case of atresia of the esophagus is as follows: A healthy mother was delivered, after an uneventful accouchement, of a seven-pound female infant. From the first nursing it was noted that vomiting of unaltered milk occurred immediately after feeding. On the fifth day of life a diagnosis of esophageal obstruction was made, and a gastrostomy performed. The infant survived the operation for seven days.

At autopsy the only pathological change of interest was found in the esophagus. From the pharynx downward, there was a fusiform dilatation which ended abruptly just above the bifurcation of the trachea. From the cardiac end of the stomach, the esophagus, of normal diameter and in normal position, proceeded to the lower surface of the pouch previously described. A probe passed from the cardiac orifice passed to within two or three millimeters of the pouch where its further progress was arrested. Fluid injected from the cardiac end, even when under considerable pressure, failed to pass the obstruction encountered by the probe.

The case of congenital bilateral hydrocele of the neck has the following history. The mother, a Russian aged 39 years, had seven spontaneous miscarriages, all of which occurred about the fifth month of gestation. Eighteen months before the birth of the present monster, she gave birth to an eight months monster identical in type with the one presently to be described, with the exception that the first monster was not as large. Both mother and father give a negative Wassermann reaction. The delivery of this monster was normal, although it occurred in the eighth month of pregnancy.

There are two types of deformity in the specimen. The least interesting abnormality is that both the upper and lower limbs are webbed. The forearm is attached to the arm by a continuous band of skin extending from the middle of the arm to the wrist. This band tightly flexes the forearm on the arm. Another band runs from the outer surface of the arm to the anterior axillary line, holding the arm tightly to the chest. The legs are flexed on the thighs by a band extending from the lower gluteal fold to about the middle of the Achilles tendon.

On the dorsolateral aspect of both sides of the neck, are two globular cystic masses which measured in the fresh condition approximately 5.5 × 4 × 3 inches. These masses extend from the middle of the scapula to the occipital protuberance, and laterally to the posterior border of the sternomastoid muscle. They are separated by a groove running along the spines of the vertebræ. The cysts contained from three to four ounces of a serous blood-stained fluid. Injection into the sacs of a solution colored with methylene blue, failed to demonstrate any connection with each other, or with the spinal axis, skull cavity, thyroid or trachea. Histologically the cysts are lined by a single layer of endothelium supported by a loose edematous connective tissue. The septum between the two cyst cavities contains a large amount of elastic tissue, and is probably the stretched ligamentum nuchæ.

Discussion:

DR. FRANK: If Dr. Rohdenburg has specific proof that the preceding monstrosity born by this mother was identical with the one shown tonight the case is of considerable importance. A large number of authorities believe that hydroceles of the neck or portions of the trunk are due to contractions of amniotic bands causing pressure upon musculature and thus producing lymph statis. It seems hardly possible that the mother would have amniotic bands occurring at the same site twice in successive pregnancies. We should have to find some other explanation for this developmental anomaly.

Dr. Moschcowitz: I performed an autopsy on a similar case, in which the upper end of the esophagus ended in a blind pouch opposite the bifurcation of the trachea, while the lower portion communicated directly with the trachea by a larger opening. This type of anomaly is by far the most common form. At that time I was unable to find an explanation of the anomaly based on embryological grounds. It is a curious fact that the common forms of congenital stenosis or atresia are at points where two tubular structures join. For example in the aorta at the entrance of the ductus arteriosus, in the duodenum at the entrance of the common bile duct, and in the intestine at Meckel's diverticulum. The fact that congenital atresia of the esophagus occurs opposite the bifurcation of the trachea could be explained in the same way.

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